



03/31/00

Customer Number: 000959

A

**DIVISIONAL-CONTINUATION APPLICATION TRANSMITTAL FORM**  
**UNDER RULE 1.53(b) (former Rule 1.60)**

DOCKET NUMBER	ANTICIPATED CLASSIFICATION OF THIS APPLICATION:		PRIOR APPLICATION SERIAL NUMBER: 08/599,226	PRIOR APPLICATION FILING DATE: APRIL 16, 1999
BBI-043CPACN	CLASS:	SUBCLASS:	EXAMINER: D. SAUNDERS	ART UNIT: 1644

ASSISTANT COMMISSIONER FOR PATENTS  
 BOX PATENT APPLICATION  
 WASHINGTON, DC 20231

jc542 U. S. PTO  
 09/540018



03/31/00

**CERTIFICATION UNDER 37 CFR 1.10**

Date of Deposit: March 31, 2000 Mailing Label Number: EL263575885US

I hereby certify that this 37 CFR 1.53(b) request and the documents referred to as attached therein are being deposited with the United States Postal Service on the date indicated above in an envelope as "Express Mail Post Office to Addressee" service under 37 CFR 1.10 and addressed to the Assistant Commissioner for Patents, Box Patent Application, Washington, D.C. 20231.

NELSON BARROS  
 Name of Person Mailing Paper

Nelson Barros  
 Signature of Person Mailing Paper

Dear Sir:

This is a request for filing a ☒ continuation ☐ divisional application under 37 CFR 1.53(b), of pending prior application serial no. 08/599,226 filed on April 16, 1999, of Jochen G. Salfeld et al., entitled HUMAN ANTIBODIES THAT BIND HUMAN TNF $\alpha$ , which is a continued prosecution application under 37 CFR 1.53(d) of serial no. 08/599,226, filed on February 9, 1996, of Jochen G. Salfeld et al., entitled HUMAN ANTIBODIES THAT BIND HUMAN TNF $\alpha$ .

1. ☒ Enclosed is a copy of the latest inventor signed application, including the oath or declaration as originally filed. The copy of the enclosed papers is as follows:

- ☒ 56 page(s) of specification (including 14 sheets of sequence listing)  
☒ 7 page(s) of claims  
☒ 1 page(s) of abstract  
☒ 10 sheet(s) of drawing (Figs. 1-8)  
☒ 21 page(s) of declaration and power of attorney.

I hereby verify that the attached papers are a true copy of the prior complete application serial no. 08/599,226 as originally filed on February 9, 1996.

2. ☐ A verified statement to establish small entity status under 37 CFR 1.9 and 1.27, a copy of which is enclosed, was filed in the prior application and such status is still proper and desired (37 CFR 1.28(a)).
3. ☒ The filing fee is calculated below:

	NUMBER OF CLAIMS FILED		NUMBER EXTRA
TOTAL	* 1	MINUS ** 20	= 0
INDEP.	* 1	MINUS *** 3	= 0
<input type="checkbox"/> MULTIPLE DEPENDENT CLAIMS			

**SMALL ENTITY**

RATE	FEE
x 9 =	\$0.00
x 39 =	\$0.00
+130 =	\$0.00
BASIC FEE	\$0.00
TOTAL	\$0.00

**OTHER THAN A SMALL ENTITY**

RATE	FEE
x 18 =	\$0.00
x 78 =	\$0.00
+260 =	\$0.00
BASIC FEE	\$690.00
TOTAL	\$690.00

OR

OR

4. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required in connection with this communication, or credit any overpayment, to Deposit Account No. 12-0080. A duplicate copy of this sheet is enclosed.
5. ☒ A check in the amount of \$ 690.00 is enclosed for payment of the filing fee.
6. ☒ Cancel in this application original claims 2-64 of the prior application before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)
7. ☐ A preliminary amendment is enclosed. (Claims added by this amendment have been properly numbered consecutively beginning with the number next following the highest numbered original claims in the prior application.)
8. ☒ Amend the specification by inserting before the first line the sentences: "This application is a continuation application of serial no. 08/599,226, filed on April 16, 1999, which is a continued prosecution application of serial no. 08/599,226, filed on February 9, 1996, pending. The contents of all of the aforementioned application(s) are hereby incorporated by reference."
9. ☐ Please abandon said prior application as of the filing date accorded this application. A duplicate copy of this transmittal is enclosed for filing in the prior application file. (May be used if signed by person authorized by §1.138 and before payment of base issue fee.)
10. ☒ Transfer the drawings from the pending prior application to this application.
11. ☐ Priority of application serial no. \_\_\_\_\_ filed on \_\_\_\_\_ in \_\_\_\_\_ is claimed under 35 U.S.C. §119.
  - ☐ The certified copy has been filed in prior application serial no. \_\_\_\_\_ filed on \_\_\_\_\_.
  - ☐ The certified copy will follow.
12. ☒ The prior application is assigned of record to BASF Aktiengesellschaft.
13. ☐ A \_\_\_\_\_ month extension of time has been submitted in the parent application Serial No. \_\_\_\_\_ in order to establish copendency with the present application.
14. ☐ Also enclosed is/are \_\_\_\_\_.
15. ☒ The power of attorney in the prior application is to Lahive & Cockfield, LLP and BASF Bioreserach Corporation
  - a. ☒ The power appears in the original papers in the prior application.
  - b. ☐ Since the power does not appear in the original papers, a copy of the power in the prior application is enclosed.
  - c. ☐ A new power has been executed and is attached.
16. ☒ Address all future communications (May only be completed by applicant, or attorney or agent of record) to Catherine J. Kara at **Customer Number: 000959** whose address is:
 

Lahive & Cockfield, LLP  
28 State Street  
Boston, Massachusetts 02109
17. ☒ Any requests for extensions of time necessary in a parent application for establishing copendency between this application and a parent application are hereby requested and the Commissioner is authorized to charge any fee associated with such an extension to Deposit Account No. 12-0080.

18. ☒ Pursuant to 37 CFR 1.821(e), the computer readable form of the sequence listing for this new application is to be identical with the computer readable form of application serial no. 08/599,226. Please use the computer readable form of application serial no. 08/599,226 in lieu of filing a duplicate computer readable form in this application. Pursuant to 37 CFR 1.821(f), the content of the paper copy of the sequence listing for this new application and the computer readable form of application serial no. 08/599,226 are the same.

March 31, 2000  
Date

LAHIVE & COCKFIELD, LLP  
28 State Street  
Boston, Massachusetts 02109  
Tel. (617) 227-7400

*Catherine J. Kara*  
Catherine J. Kara

Reg. No. 41,106

☐ inventor(s) ☐ filed under §1.34(a)

☐ assignee of complete interest

☒ attorney or agent of record

HUMAN ANTIBODIES THAT BIND HUMAN TNF $\alpha$ **Background of the Invention**

Tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) is a cytokine produced by numerous cell types, including monocytes and macrophages, that was originally identified based on its capacity to induce the necrosis of certain mouse tumors (see e.g., Old, L. (1985) *Science* 230:630-632). Subsequently, a factor termed cachectin, associated with cachexia, was shown to be the same molecule as TNF $\alpha$ . TNF $\alpha$  has been implicated in mediating shock (see e.g., Beutler, B. and Cerami, A. (1988) *Annu. Rev. Biochem.* 57:505-518; Beutler, B. and Cerami, A. (1989) *Annu. Rev. Immunol.* 7:625-655). Furthermore, TNF $\alpha$  has been implicated in the pathophysiology of a variety of other human diseases and disorders, including sepsis, infections, autoimmune diseases, transplant rejection and graft-versus-host disease (see e.g., Vassili, P. (1992) *Annu. Rev. Immunol.* 10:411-452; Tracey, K.J. and Cerami, A. (1994) *Annu. Rev. Med.* 45:491-503).

Because of the harmful role of human TNF $\alpha$  (hTNF $\alpha$ ) in a variety of human disorders, therapeutic strategies have been designed to inhibit or counteract hTNF $\alpha$  activity. In particular, antibodies that bind to, and neutralize, hTNF $\alpha$  have been sought as a means to inhibit hTNF $\alpha$  activity. Some of the earliest of such antibodies were mouse monoclonal antibodies (mAbs), secreted by hybridomas prepared from lymphocytes of mice immunized with hTNF $\alpha$  (see e.g., Hahn T; et al., (1985) *Proc Natl Acad Sci USA* 82: 3814-3818; Liang, C-M., et al. (1986) *Biochem. Biophys. Res. Commun.* 137:847-854; Hirai, M., et al. (1987) *J. Immunol. Methods* 96:57-62; Fendly, B.M., et al. (1987) *Hybridoma* 6:359-370; Möller, A., et al. (1990) *Cytokine* 2:162-169; U.S. Patent No. 5,231,024 to Moeller et al.; European Patent Publication No. 186 833 B1 by Wallach, D.; European Patent Application Publication No. 218 868 A1 by Old et al.; European Patent Publication No. 260 610 B1 by Moeller, A., et al.). While these mouse anti-hTNF $\alpha$  antibodies often displayed high affinity for hTNF $\alpha$  (e.g., Kd  $\leq 10^{-9}$ M) and were able to neutralize hTNF $\alpha$  activity, their use *in vivo* may be limited by problems associated with administration of mouse antibodies to humans, such as short serum half life, an inability to trigger certain human effector functions and elicitation of an unwanted immune response against the mouse antibody in a human (the "human anti-mouse antibody" (HAMA) reaction).

In an attempt to overcome the problems associated with use of fully-murine antibodies in humans, murine anti-hTNF $\alpha$  antibodies have been genetically engineered to be more "human-like." For example, chimeric antibodies, in which the variable regions of the antibody chains are murine-derived and the constant regions of the antibody chains are human-derived, have been prepared (Knight, D.M., et al. (1993) *Mol. Immunol.* 30:1443-1453; PCT Publication No. WO 92/16553 by Daddona, P.E., et al.). Additionally, humanized antibodies, in which the hypervariable domains of the antibody variable regions are murine-derived but the remainder of the variable regions and the antibody constant

regions are human-derived, have also been prepared (PCT Publication No. WO 92/11383 by Adair, J.R., *et al.*). However, because these chimeric and humanized antibodies still retain some murine sequences, they still may elicit an unwanted immune reaction, the human anti-chimeric antibody (HACA) reaction, especially when administered for prolonged periods, *e.g.*, for chronic indications, such as rheumatoid arthritis (see *e.g.*, Elliott, M.J., *et al.* (1994) *Lancet* **344**:1125-1127; Elliot, M.J., *et al.* (1994) *Lancet* **344**:1105-1110).

A preferred hTNF $\alpha$  inhibitory agent to murine mAbs or derivatives thereof (*e.g.*, chimeric or humanized antibodies) would be an entirely human anti-hTNF $\alpha$  antibody, since such an agent should not elicit the HAMA reaction, even if used for prolonged periods.

Human monoclonal autoantibodies against hTNF $\alpha$  have been prepared using human hybridoma techniques (Boyle, P., *et al.* (1993) *Cell. Immunol.* **152**:556-568; Boyle, P., *et al.* (1993) *Cell. Immunol.* **152**:569-581; European Patent Application Publication No. 614 984 A2 by Boyle, *et al.*). However, these hybridoma-derived monoclonal autoantibodies were reported to have an affinity for hTNF $\alpha$  that was too low to calculate by conventional methods, were unable to bind soluble hTNF $\alpha$  and were unable to neutralize hTNF $\alpha$ -induced cytotoxicity (see Boyle, *et al.*; *supra*). Moreover, the success of the human hybridoma technique depends upon the natural presence in human peripheral blood of lymphocytes producing autoantibodies specific for hTNF $\alpha$ . Certain studies have detected serum autoantibodies against hTNF $\alpha$  in human subjects (Fomsgaard, A., *et al.* (1989) *Scand. J. Immunol.* **30**:219-223; Bendtzen, K., *et al.* (1990) *Prog. Leukocyte Biol.* **10B**:447-452), whereas others have not (Leusch, H-G., *et al.* (1991) *J. Immunol. Methods* **139**:145-147).

Alternative to naturally-occurring human anti-hTNF $\alpha$  antibodies would be a recombinant hTNF $\alpha$  antibody. Recombinant human antibodies that bind hTNF $\alpha$  with relatively low affinity (*i.e.*,  $K_d \sim 10^{-7}M$ ) and a fast off rate (*i.e.*,  $K_{off} \sim 10^{-2} \text{ sec}^{-1}$ ) have been described (Griffiths, A.D., *et al.* (1993) *EMBO J.* **12**:725-734). However, because of their relatively fast dissociation kinetics, these antibodies may not be suitable for therapeutic use. Additionally, a recombinant human anti-hTNF $\alpha$  has been described that does not neutralize hTNF $\alpha$  activity, but rather enhances binding of hTNF $\alpha$  to the surface of cells and enhances internalization of hTNF $\alpha$  (Lidbury, A., *et al.* (1994) *Biotechnol. Ther.* **5**:27-45; PCT Publication No. WO 92/03145 by Aston, R. *et al.*)

Accordingly, human antibodies, such as recombinant human antibodies, that bind soluble hTNF $\alpha$  with high affinity and slow dissociation kinetics and that have the capacity to neutralize hTNF $\alpha$  activity, including hTNF $\alpha$ -induced cytotoxicity (*in vitro* and *in vivo*) and hTNF $\alpha$ -induced cell activation, are still needed.

### **Summary of the Invention**

This invention provides human antibodies, preferably recombinant human antibodies, that specifically bind to human TNF $\alpha$ . The antibodies of the invention are characterized by binding to hTNF $\alpha$  with high affinity and slow dissociation kinetics and by neutralizing

hTNF $\alpha$  activity, including hTNF $\alpha$ -induced cytotoxicity (*in vitro* and *in vivo*) and hTNF $\alpha$ -induced cellular activation. The antibodies can be full-length (e.g., an IgG1 or IgG4 antibody) or can comprise only an antigen-binding portion (e.g., a Fab, F(ab')<sub>2</sub> or scFv fragment). The most preferred recombinant antibody of the invention, termed D2E7, has a light chain CDR3 domain comprising the amino acid sequence of SEQ ID NO: 3 and a heavy chain CDR3 domain comprising the amino acid sequence of SEQ ID NO: 4. Preferably, the D2E7 antibody has a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO: 1 and a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 2.

In one embodiment, the invention provides an isolated human antibody, or an antigen-binding portion thereof, that dissociates from human TNF $\alpha$  with a  $K_d$  of  $1 \times 10^{-8}$  M or less and a  $K_{off}$  rate constant of  $1 \times 10^{-3}$  s<sup>-1</sup> or less, both determined by surface plasmon resonance, and neutralizes human TNF $\alpha$  cytotoxicity in a standard *in vitro* L929 assay with an IC<sub>50</sub> of  $1 \times 10^{-7}$  M or less. More preferably, the isolated human antibody, or antigen-binding portion thereof, dissociates from human TNF $\alpha$  with a  $K_{off}$  of  $5 \times 10^{-4}$  s<sup>-1</sup> or less, or even more preferably, with a  $K_{off}$  of  $1 \times 10^{-4}$  s<sup>-1</sup> or less. More preferably, the isolated human antibody, or antigen-binding portion thereof, neutralizes human TNF $\alpha$  cytotoxicity in a standard *in vitro* L929 assay with an IC<sub>50</sub> of  $1 \times 10^{-8}$  M or less, even more preferably with an IC<sub>50</sub> of  $1 \times 10^{-9}$  M or less and still more preferably with an IC<sub>50</sub> of  $1 \times 10^{-10}$  M or less.

In another embodiment, the invention provides a human antibody, or antigen-binding portion thereof, with the following characteristics:

a) dissociates from human TNF $\alpha$  with a  $K_{off}$  of  $1 \times 10^{-3}$  s<sup>-1</sup> or less, as determined by surface plasmon resonance;

b) has a light chain CDR3 domain comprising the amino acid sequence of SEQ ID NO: 3, or modified from SEQ ID NO: 3 by a single alanine substitution at position 1, 4, 5, 7 or 8 or by one to five conservative amino acid substitutions at positions 1, 3, 4, 6, 7, 8 and/or 9;

c) has a heavy chain CDR3 domain comprising the amino acid sequence of SEQ ID NO: 4, or modified from SEQ ID NO: 4 by a single alanine substitution at position 2, 3, 4, 5, 6, 8, 9, 10 or 11 or by one to five conservative amino acid substitutions at positions 2, 3, 4, 5, 6, 8, 9, 10, 11 and/or 12.

More preferably, the antibody, or antigen-binding portion thereof, dissociates from human TNF $\alpha$  with a  $K_{off}$  of  $5 \times 10^{-4}$  s<sup>-1</sup> or less. Still more preferably, the antibody, or antigen-binding portion thereof, dissociates from human TNF $\alpha$  with a  $K_{off}$  of  $1 \times 10^{-4}$  s<sup>-1</sup> or less.

In yet another embodiment, the invention provides a human antibody, or an antigen-binding portion thereof, with an LCVR having CDR3 domain comprising the amino acid sequence of SEQ ID NO: 3, or modified from SEQ ID NO: 3 by a single alanine substitution at position 1, 4, 5, 7 or 8, and with an HCVR having a CDR3 domain comprising the amino

acid sequence of SEQ ID NO: 4, or modified from SEQ ID NO: 4 by a single alanine substitution at position 2, 3, 4, 5, 6, 8, 9, 10 or 11. More preferably, the LCVR further has a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 5 and the HCVR further has a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 6. Still more preferably, the LCVR further has CDR1 domain comprising the amino acid sequence of SEQ ID NO: 7 and the HCVR has a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 8.

In still another embodiment, the invention provides an isolated human antibody, or an antigen binding portion thereof, with an LCVR comprising the amino acid sequence of SEQ ID NO: 1 and an HCVR comprising the amino acid sequence of SEQ ID NO: 2. In certain embodiments, the antibody has an IgG1 heavy chain constant region or an IgG4 heavy chain constant region. In yet other embodiments, the antibody is a Fab fragment, an F(ab')<sub>2</sub> fragment or a single chain Fv fragment.

In still other embodiments, the invention provides antibodies, or antigen-binding portions thereof, with an LCVR having CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26 or with an HCVR having a CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34 and SEQ ID NO: 35.

In yet another embodiment, the invention provides an isolated human antibody, or antigen-binding portion thereof, that neutralizes the activity of human TNF $\alpha$ , chimpanzee TNF $\alpha$  and at least one additional primate TNF $\alpha$  selected from the group consisting of baboon TNF $\alpha$ , marmoset TNF $\alpha$ , cynomolgus TNF $\alpha$  and rhesus TNF $\alpha$ . In one subembodiment, the isolated human antibody, or antigen-binding portion thereof, also neutralizes the activity of mouse TNF $\alpha$ . In another subembodiment, the isolated human antibody, or antigen-binding portion thereof, also neutralizes the activity of pig TNF $\alpha$ .

Another aspect of the invention pertains to nucleic acid molecules encoding the antibodies, or antigen-binding portions, of the invention. A preferred nucleic acid of the invention, encoding a D2E7 LCVR, has the nucleotide sequence shown in Figure 7 and SEQ ID NO 36. Another preferred nucleic acid of the invention, encoding a D2E7 HCVR, has the nucleotide sequence shown in Figure 8 and SEQ ID NO 37. Recombinant expression vectors carrying the antibody-encoding nucleic acids of the invention, and host cells into which such vectors have been introduced, are also encompassed by the invention, as are methods of making the antibodies of the invention by culturing the host cells of the invention.

Yet another aspect of the invention pertains to methods for inhibiting human TNF $\alpha$  activity using an antibody, or antigen-binding portion thereof, of the invention. In one

embodiment, the method comprises contacting human TNF $\alpha$  with the antibody of the invention, or antigen-binding portion thereof, such that human TNF $\alpha$  activity is inhibited. In another embodiment, the method comprises administering an antibody of the invention, or antigen-binding portion thereof, to a human subject suffering from a disorder in which TNF $\alpha$  activity is detrimental such that human TNF $\alpha$  activity in the human subject is inhibited. The disorder can be, for example, sepsis, an autoimmune disease (e.g., rheumatoid arthritis, allergy, multiple sclerosis, autoimmune diabetes, autoimmune uveitis and nephrotic syndrome), an infectious disease, a malignancy, transplant rejection or graft-versus-host disease, a pulmonary disorder, a bone disorder, an intestinal disorder or a cardiac disorder.

### **Brief Description of the Drawings**

Figures 1A and 1B show the amino acid sequences of the light chain variable region of D2E7 (D2E7 VL; also shown in SEQ ID NO: 1), alanine-scan mutants of D2E7 VL (LD2E7\*.A1, LD2E7\*.A3, LD2E7\*.A4, LD2E7\*.A5, LD2E7\*.A7 and LD2E7\*.A8), the light chain variable region of the D2E7-related antibody 2SD4 (2SD4 VL; also shown in SEQ ID NO: 9) and other D2E7-related light chain variable regions (EP B12, VL10E4, VL100A9, VL100D2, VL10F4, LOE5, VLLOF9, VLL0F10, VLLOG7, VLLOG9, VLLOH1, VLLOH10, VL1B7, VL1C1, VL1C7, VL0.1F4, VL0.1H8, LOE7, LOE7.A and LOE7.T). Figure 1A shows the FR1, CDR1, FR2 and CDR2 domains. Figure 1B shows the FR3, CDR3 and FR4 domains. The light chain CDR1 ("CDR L1"), CDR2 ("CDR L2") and CDR3 ("CDR L3") domains are boxed.

Figures 2A and 2B show the amino acid sequences of the heavy chain variable region of D2E7 (D2E7 VH; also shown in SEQ ID NO: 2), alanine-scan mutants of D2E7 VH (HD2E7\*.A1, HD2E7\*.A2, HD2E7\*.A3, HD2E7\*.A4, HD2E7\*.A5, HD2E7\*.A6, HD2E7\*.A7, HD2E7\*.A8 and HD2E7\*.A9), the heavy chain variable region of the D2E7-related antibody 2SD4 (2SD4 VH; also shown in SEQ ID NO: 10) and other D2E7-related heavy chain variable regions (VH1B11, VH1D8, VH1A11, VH1B12, VH1-D2, VH1E4, VH1F6, VH1G1, 3C-H2, VH1-D2.N and VH1-D2.Y). Figure 2A shows the FR1, CDR1, FR2 and CDR2 domains. Figure 2B shows the FR3, CDR3 and FR4 domains. The heavy chain CDR1 ("CDR H1"), CDR2 ("CDR H2") and CDR3 ("CDR H3") domains are boxed.

Figure 3 is a graph depicting the inhibition of TNF $\alpha$ -induced L929 cytotoxicity by the human anti-hTNF $\alpha$  antibody D2E7, as compared to the murine anti-hTNF $\alpha$  antibody MAK 195 (IgG1 or Fab fragment).

Figure 4 is a graph depicting the inhibition of rhTNF $\alpha$  binding to hTNF $\alpha$  receptors on U-937 cells by the human anti-hTNF $\alpha$  antibody D2E7, as compared to the murine anti-hTNF $\alpha$  antibody MAK 195 (IgG1 or Fab fragment).

Figure 5 is a graph depicting the inhibition of TNF $\alpha$ -induced ELAM-1 expression on HUVEC by the human anti-hTNF $\alpha$  antibody D2E7, as compared to the murine anti-hTNF $\alpha$  antibody MAK 195 (IgG1 or Fab fragment).



Figure 6 is a bar graph depicting protection from TNF $\alpha$ -induced lethality in D-galactosamine-sensitized mice by administration of the human anti-hTNF $\alpha$  antibody D2E7 (black bars), as compared to the murine anti-hTNF $\alpha$  antibody MAK 195 (hatched bars).

Figure 7 shows the nucleotide sequence of the light chain variable region of D2E7, with the predicted amino acid sequence below the nucleotide sequence. The CDR L1, CDR L2 and CDR L3 regions are underlined.

Figure 8 shows the nucleotide sequence of the heavy chain variable region of D2E7, with the predicted amino acid sequence below the nucleotide sequence. The CDR H1, CDR H2 and CDR H3 regions are underlined.

### **Detailed Description of the Invention**

This invention pertains to isolated human antibodies, or antigen-binding portions thereof, that bind to human TNF $\alpha$  with high affinity, a low off rate and high neutralizing capacity. Various aspects of the invention relate to antibodies and antibody fragments, and pharmaceutical compositions thereof, as well as nucleic acids, recombinant expression vectors and host cells for making such antibodies and fragments. Methods of using the antibodies of the invention to detect human TNF $\alpha$  or to inhibit human TNF $\alpha$  activity, either *in vitro* or *in vivo*, are also encompassed by the invention.

In order that the present invention may be more readily understood, certain terms are first defined.

The term "human TNF $\alpha$ " (abbreviated herein as hTNF $\alpha$ , or simply hTNF), as used herein, is intended to refer to a human cytokine that exists as a 17 kD secreted form and a 26 kD membrane associated form, the biologically active form of which is composed of a trimer of noncovalently bound 17 kD molecules. The structure of hTNF $\alpha$  is described further in, for example, Pennica, D., *et al.* (1984) *Nature* 312:724-729; Davis, J.M., *et al.* (1987) *Biochemistry* 26:1322-1326; and Jones, E.Y., *et al.* (1989) *Nature* 338:225-228. The term human TNF $\alpha$  is intended to include recombinant human TNF $\alpha$  (rhTNF $\alpha$ ), which can be prepared by standard recombinant expression methods or purchased commercially (R & D Systems, Catalog No. 210-TA, Minneapolis, MN).

The term "antibody", as used herein, is intended to refer to immunoglobulin molecules comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or VL) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs

and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

The term "antigen-binding portion" of an antibody (or simply "antibody portion"), as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen (e.g., hTNF $\alpha$ ). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab')<sub>2</sub> fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody; (v) a dAb fragment (Ward *et al.*, (1989) *Nature* 341:544-546), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see e.g., Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. Other forms of single chain antibodies, such as diabodies are also encompassed. Diabodies are bivalent, bispecific antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see e.g., Holliger, P., *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:6444-6448; Poljak, R.J., *et al.* (1994) *Structure* 2:1121-1123).

Still further, an antibody or antigen-binding portion thereof may be part of a larger immunoadhesion molecules, formed by covalent or noncovalent association of the antibody or antibody portion with one or more other proteins or peptides. Examples of such immunoadhesion molecules include use of the streptavidin core region to make a tetrameric scFv molecule (Kipriyanov, S.M., *et al.* (1995) *Human Antibodies and Hybridomas* 6:93-101) and use of a cysteine residue, a marker peptide and a C-terminal polyhistidine tag to make bivalent and biotinylated scFv molecules (Kipriyanov, S.M., *et al.* (1994) *Mol. Immunol.* 31:1047-1058). Antibody portions, such as Fab and F(ab')<sub>2</sub> fragments, can be prepared from whole antibodies using conventional techniques, such as papain or pepsin digestion, respectively, of whole antibodies. Moreover, antibodies, antibody portions and immunoadhesion molecules can be obtained using standard recombinant DNA techniques, as described herein.

The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs and in particular CDR3. However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

The term "recombinant human antibody", as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell (described further in Section II, below), antibodies isolated from a recombinant, combinatorial human antibody library (described further in Section III, below), antibodies isolated from an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes (see e.g., Taylor, L.D., et al. (1992) *Nucl. Acids Res.* 20:6287-6295) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies are subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

An "isolated antibody", as used herein, is intended to refer to an antibody that is substantially free of other antibodies having different antigenic specificities (e.g., an isolated antibody that specifically binds hTNF $\alpha$  is substantially free of antibodies that specifically bind antigens other than hTNF $\alpha$ ). An isolated antibody that specifically binds hTNF $\alpha$  may, however, have cross-reactivity to other antigens, such as TNF $\alpha$  molecules from other species (discussed in further detail below). Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

A "neutralizing antibody", as used herein (or an "antibody that neutralized hTNF $\alpha$  activity"), is intended to refer to an antibody whose binding to hTNF $\alpha$  results in inhibition of the biological activity of hTNF $\alpha$ . This inhibition of the biological activity of hTNF $\alpha$  can be assessed by measuring one or more indicators of hTNF $\alpha$  biological activity, such as hTNF $\alpha$ -induced cytotoxicity (either *in vitro* or *in vivo*), hTNF $\alpha$ -induced cellular activation and hTNF $\alpha$  binding to hTNF $\alpha$  receptors. These indicators of hTNF $\alpha$  biological activity can be assessed by one or more of several standard *in vitro* or *in vivo* assays known in the art (see Example 4). Preferably, the ability of an antibody to neutralize hTNF $\alpha$  activity is assessed

by inhibition of hTNF $\alpha$ -induced cytotoxicity of L929 cells. As an additional or alternative parameter of hTNF $\alpha$  activity, the ability of an antibody to inhibit hTNF $\alpha$ -induced expression of ELAM-1 on HUVEC, as a measure of hTNF $\alpha$ -induced cellular activation, can be assessed.

- The term "surface plasmon resonance", as used herein, refers to an optical
- 5 phenomenon that allows for the analysis of real-time biospecific interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIAcore system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, NJ). For further descriptions, see Example 1 and Jönsson, U., *et al.* (1993) *Ann. Biol. Clin.* **51**:19-26; Jönsson, U., *et al.* (1991) *Biotechniques* **11**:620-627; Johnsson, B., *et al.* (1995) *J. Mol. Recognit.* **8**:125-131; and Johnsson, B., *et al.* (1991) *Anal. Biochem.* **198**:268-277.

The term " $K_{off}$ ", as used herein, is intended to refer to the off rate constant for dissociation of an antibody from the antibody/antigen complex.

The term " $K_d$ ", as used herein, is intended to refer to the dissociation constant of a particular antibody-antigen interaction.

- 15 The term "nucleic acid molecule", as used herein, is intended to include DNA molecules and RNA molecules. A nucleic acid molecule may be single-stranded or double-stranded, but preferably is double-stranded DNA.

- The term "isolated nucleic acid molecule", as used herein in reference to nucleic acids encoding antibodies or antibody portions (*e.g.*, VH, VL, CDR3) that bind hTNF $\alpha$ , is intended
- 20 to refer to a nucleic acid molecule in which the nucleotide sequences encoding the antibody or antibody portion are free of other nucleotide sequences encoding antibodies or antibody portions that bind antigens other than hTNF $\alpha$ , which other sequences may naturally flank the nucleic acid in human genomic DNA. Thus, for example, an isolated nucleic acid of the invention encoding a VH region of an anti-TNF $\alpha$  antibody contains no other sequences
- 25 encoding other VH regions that bind antigens other than TNF $\alpha$ .

- The term "vector", as used herein, is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional
- 30 DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome.
- 35 Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of

vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The term "recombinant host cell" (or simply "host cell"), as used herein, is intended to refer to a cell into which a recombinant expression vector has been introduced. It should be understood that such terms are intended to refer not only to the particular subject cell but to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein.

Various aspects of the invention are described in further detail in the following subsections.

#### I. Human Antibodies that Bind Human TNF $\alpha$

This invention provides isolated human antibodies, or antigen-binding portions thereof, that bind to human TNF $\alpha$  with high affinity, a low off rate and high neutralizing capacity. Preferably, the human antibodies of the invention are recombinant, neutralizing human anti-hTNF $\alpha$  antibodies. The most preferred recombinant, neutralizing antibody of the invention is referred to herein as D2E7 and has VL and VH sequences as shown in Figure 1A, 1B and Figure 2A, 2B, respectively (the amino acid sequence of the D2E7 VL region is also shown in SEQ ID NO: 1; the amino acid sequence of the D2E7 VH region is also shown in SEQ ID NO: 2). The binding properties of D2E7, as compared to the murine anti-hTNF $\alpha$  MAK 195 mAb that exhibits high affinity and slow dissociation kinetics and another human anti-hTNF $\alpha$  antibody related in sequence to D2E7, 2SD4, are summarized below:

Antibody	$K_{off}$ sec <sup>-1</sup>	t1/2 minutes	$k_{on}$ M <sup>-1</sup> sec <sup>-1</sup>	$K_d^*$ M	Stoichio- metry	$K_d^{**}$ M
D2E7 IgG4	$9.9 \times 10^{-5}$	117	$3.8 \times 10^5$	$2.6 \times 10^{-10}$	1.2	$3.4 \times 10^{-10}$
2SD4 IgG4	$8.4 \times 10^{-3}$	1	$4.2 \times 10^5$	$2.0 \times 10^{-8}$	0.8	$4.8 \times 10^{-10}$
MAK 195 IgG1	$8.2 \times 10^{-5}$	141	$1.6 \times 10^5$	$5.1 \times 10^{-10}$	1.2	$1.7 \times 10^{-10}$
MAK 195 F(ab') <sub>2</sub>	$8.7 \times 10^{-5}$	133	$1.9 \times 10^5$	$4.6 \times 10^{-10}$	1.4	$2.2 \times 10^{-10}$

\*  $K_d = K_{off}/k_{on}$  (kinetic analysis by surface plasmon resonance as described in Example 1)

\*\*  $K_d = [Ab][TNF\alpha]/[Ab:TNF\alpha]$  (equilibrium analysis)

The D2E7 antibody, and related antibodies, also exhibit a strong capacity to neutralize hTNF $\alpha$  activity, as assessed by several *in vitro* and *in vivo* assays (see Example 4). For example, these antibodies neutralize hTNF $\alpha$ -induced cytotoxicity of L929 cells with IC<sub>50</sub> values in the range of 10<sup>-7</sup> M to 10<sup>-11</sup> M. D2E7, when expressed as a full-length IgG4 antibody, neutralizes hTNF $\alpha$ -induced cytotoxicity of L929 cells with IC<sub>50</sub> of 4.7 x 10<sup>-11</sup> M.

Moreover, the neutralizing capacity of D2E7 is maintained when the antibody is expressed as a Fab, F(ab')<sub>2</sub> or scFv fragment. D2E7 also inhibits TNF $\alpha$ -induced cellular activation, as measured by hTNF $\alpha$ -induced ELAM-1 expression on HUVEC (IC<sub>50</sub> = 6  $\times$  10<sup>-11</sup> M), and binding of hTNF $\alpha$  to hTNF $\alpha$  receptors on U-937 cells (IC<sub>50</sub> = 3  $\times$  10<sup>-10</sup> M). Regarding the latter, D2E7 inhibits the binding of hTNF $\alpha$  to both the p60 and p80 hTNF $\alpha$  receptors. Furthermore, the antibody inhibits hTNF $\alpha$ -induced lethality *in vivo* in mice (ED<sub>50</sub> = 1-2.5  $\mu$ g/mouse).

Regarding the binding specificity of D2E7, this antibody binds to human TNF $\alpha$  in various forms, including soluble hTNF $\alpha$ , transmembrane hTNF $\alpha$  and hTNF $\alpha$  bound to cellular receptors. D2E7 does not specifically bind to other cytokines, such as lymphotoxin (TNF $\beta$ ), IL-1 $\alpha$ , IL-1 $\beta$ , IL-2, IL-4, IL-6, IL-8, IFN $\gamma$  and TGF $\beta$ . However, D2E7 does exhibit crossreactivity to tumor necrosis factors from other species. For example, the antibody neutralizes the activity of at least five primate TNF $\alpha$ s (chimpanzee, baboon, marmoset, cynomolgus and rhesus) with approximately equivalent IC<sub>50</sub> values as for neutralization of hTNF $\alpha$  (see Example 4, subsection E). D2E7 also neutralizes the activity of mouse TNF $\alpha$ , although approximately 1000-fold less well than human TNF $\alpha$  (see Example 4, subsection E). D2E7 also binds to porcine TNF $\alpha$ .

In one aspect, the invention pertains to D2E7 antibodies and antibody portions, D2E7-related antibodies and antibody portions, and other human antibodies and antibody portions with equivalent properties to D2E7, such as high affinity binding to hTNF $\alpha$  with low dissociation kinetics and high neutralizing capacity. In one embodiment, the invention provides an isolated human antibody, or an antigen-binding portion thereof, that dissociates from human TNF $\alpha$  with a K<sub>d</sub> of 1  $\times$  10<sup>-8</sup> M or less and a K<sub>off</sub> rate constant of 1  $\times$  10<sup>-3</sup> s<sup>-1</sup> or less, both determined by surface plasmon resonance, and neutralizes human TNF $\alpha$  cytotoxicity in a standard *in vitro* L929 assay with an IC<sub>50</sub> of 1  $\times$  10<sup>-7</sup> M or less. More preferably, the isolated human antibody, or antigen-binding portion thereof, dissociates from human TNF $\alpha$  with a K<sub>off</sub> of 5  $\times$  10<sup>-4</sup> s<sup>-1</sup> or less, or even more preferably, with a K<sub>off</sub> of 1  $\times$  10<sup>-4</sup> s<sup>-1</sup> or less. More preferably, the isolated human antibody, or antigen-binding portion thereof, neutralizes human TNF $\alpha$  cytotoxicity in a standard *in vitro* L929 assay with an IC<sub>50</sub> of 1  $\times$  10<sup>-8</sup> M or less, even more preferably with an IC<sub>50</sub> of 1  $\times$  10<sup>-9</sup> M or less and still more preferably with an IC<sub>50</sub> of 1  $\times$  10<sup>-10</sup> M or less. In a preferred embodiment, the antibody is an isolated human recombinant antibody, or an antigen-binding portion thereof. In another preferred embodiment, the antibody also neutralizes TNF $\alpha$ -induced cellular activation, as assessed using a standard *in vitro* assay for TNF $\alpha$ -induced ELAM-1 expression on human umbilical vein endothelial cells (HUVEC).

Surface plasmon resonance analysis for determining K<sub>d</sub> and K<sub>off</sub> can be performed as described in Example 1. A standard *in vitro* L929 assay for determining IC<sub>50</sub> values is described in Example 4, subsection A. A standard *in vitro* assay for TNF $\alpha$ -induced ELAM-1 expression on human umbilical vein endothelial cells (HUVEC) is described in Example 4,

subsection C. Examples of recombinant human antibodies that meet, or are predicted to meet, the aforementioned kinetic and neutralization criteria include antibodies having the following [VH/VL] pairs, the sequences of which are shown in Figures 1A, 1B, 2A and 2B (see also Examples 2, 3 and 4 for kinetic and neutralization analyses): [D2E7 VH/D2E7 VL];

- 5 [HD2E7\*.A1/D2E7 VL], [HD2E7\*.A2/D2E7 VL], [HD2E7\*.A3/D2E7 VL], [HD2E7\*.A4/D2E7 VL], [HD2E7\*.A5/D2E7 VL], [HD2E7\*.A6/D2E7 VL], [HD2E7\*.A7/D2E7 VL], [HD2E7\*.A8/D2E7 VL], [HD2E7\*.A9/D2E7 VL], [D2E7 VH/LD2E7\*.A1], [D2E7 VH/LD2E7\*.A4], [D2E7 VH/LD2E7\*.A5], [D2E7 VH/LD2E7\*.A7], [D2E7 VH/LD2E7\*.A8], [HD2E7\*.A9/LD2E7\*.A1], [VH1-D2/LOE7],
- 10 [VH1-D2.N/LOE7.T], [VH1-D2.Y/LOE7.A], [VH1-D2.N/LOE7.A], [VH1-D2/EP B12] and [3C-H2/LOE7].

It is well known in the art that antibody heavy and light chain CDR3 domains play an important role in the binding specificity/affinity of an antibody for an antigen. Accordingly, in another aspect, the invention pertains to human antibodies that have slow dissociation kinetics for association with hTNF $\alpha$  and that have light and heavy chain CDR3 domains that structurally are identical to or related to those of D2E7. As demonstrated in Example 3, position 9 of the D2E7 VL CDR3 can be occupied by Ala or Thr without substantially affecting the  $K_{off}$ . Accordingly, a consensus motif for the D2E7 VL CDR3 comprises the amino acid sequence: Q-R-Y-N-R-A-P-Y-(T/A) (SEQ ID NO: 3). Additionally, position 12 of the D2E7 VH CDR3 can be occupied by Tyr or Asn, without substantially affecting the  $K_{off}$ . Accordingly, a consensus motif for the D2E7 VH CDR3 comprises the amino acid sequence: V-S-Y-L-S-T-A-S-S-L-D-(Y/N) (SEQ ID NO: 4). Moreover, as demonstrated in Example 2, the CDR3 domain of the D2E7 heavy and light chains is amenable to substitution with a single alanine residue (at position 1, 4, 5, 7 or 8 within the VL CDR3 or at position 2, 3, 4, 5, 6, 8, 9, 10 or 11 within the VH CDR3) without substantially affecting the  $K_{off}$ . Still further, the skilled artisan will appreciate that, given the amenability of the D2E7 VL and VH CDR3 domains to substitutions by alanine, substitution of other amino acids within the CDR3 domains may be possible while still retaining the low off rate constant of the antibody, in particular substitutions with conservative amino acids. A "conservative amino acid substitution", as used herein, is one in which one amino acid residue is replaced with another amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Preferably, no more than one to five conservative amino acid substitutions are made within the D2E7 VL and/or VH CDR3 domains. More preferably, no more than one to three conservative amino

acid substitutions are made within the D2E7 VL and/or VH CDR3 domains. Additionally, conservative amino acid substitutions should not be made at amino acid positions critical for binding to hTNF $\alpha$ . As shown in Example 3, positions 2 and 5 of the D2E7 VL CDR3 and positions 1 and 7 of the D2E7 VH CDR3 appear to be critical for interaction with hTNF $\alpha$  and thus, conservative amino acid substitutions preferably are not made at these positions (although an alanine substitution at position 5 of the D2E7 VL CDR3 is acceptable, as described above).

Accordingly, in another embodiment, the invention provides an isolated human antibody, or antigen-binding portion thereof, with the following characteristics:

a) dissociates from human TNF $\alpha$  with a  $K_{off}$  rate constant of  $1 \times 10^{-3} \text{ s}^{-1}$  or less, as determined by surface plasmon resonance;

b) has a light chain CDR3 domain comprising the amino acid sequence of SEQ ID NO: 3, or modified from SEQ ID NO: 3 by a single alanine substitution at position 1, 4, 5, 7 or 8 or by one to five conservative amino acid substitutions at positions 1, 3, 4, 6, 7, 8 and/or 9;

c) has a heavy chain CDR3 domain comprising the amino acid sequence of SEQ ID NO: 4, or modified from SEQ ID NO: 4 by a single alanine substitution at position 2, 3, 4, 5, 6, 8, 9, 10 or 11 or by one to five conservative amino acid substitutions at positions 2, 3, 4, 5, 6, 8, 9, 10, 11 and/or 12.

More preferably, the antibody, or antigen-binding portion thereof, dissociates from human TNF $\alpha$  with a  $K_{off}$  of  $5 \times 10^{-4} \text{ s}^{-1}$  or less. Even more preferably, the antibody, or antigen-binding portion thereof, dissociates from human TNF $\alpha$  with a  $K_{off}$  of  $1 \times 10^{-4} \text{ s}^{-1}$  or less.

In yet another embodiment, the invention provides an isolated human antibody, or an antigen-binding portion thereof, with a light chain variable region (LCVR) having a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 3, or modified from SEQ ID NO: 3 by a single alanine substitution at position 1, 4, 5, 7 or 8, and with a heavy chain variable region (HCVR) having a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 4, or modified from SEQ ID NO: 4 by a single alanine substitution at position 2, 3, 4, 5, 6, 8, 9, 10 or 11. Preferably, the LCVR further has a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 5 (*i.e.*, the D2E7 VL CDR2) and the HCVR further has a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 6 (*i.e.*, the D2E7 VH CDR2). Even more preferably, the LCVR further has CDR1 domain comprising the amino acid sequence of SEQ ID NO: 7 (*i.e.*, the D2E7 VL CDR1) and the HCVR has a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 8 (*i.e.*, the D2E7 VH CDR1). The framework regions for VL preferably are from the V $\kappa$ 1 human germline family, more preferably from the A20 human germline V $\kappa$  gene and most preferably from the D2E7 VL framework sequences shown in Figures 1A and 1B. The framework regions for VH preferably are from the V $\text{H}3$  human germline family, more preferably from the DP-31 human



germline VH gene and most preferably from the D2E7 VH framework sequences shown in Figures 2A and 2B.

5 In still another embodiment, the invention provides an isolated human antibody, or an antigen binding portion thereof, with a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO: 1 (*i.e.*, the D2E7 VL) and a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 2 (*i.e.*, the D2E7 VH). In certain embodiments, the antibody comprises a heavy chain constant region, such as an IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM or IgD constant region. Preferably, the heavy chain  
10 constant region is an IgG1 heavy chain constant region or an IgG4 heavy chain constant region. Furthermore, the antibody can comprise a light chain constant region, either a kappa light chain constant region or a lambda light chain constant region. Preferably, the antibody comprises a kappa light chain constant region. Alternatively, the antibody portion can be, for example, a Fab fragment or a single chain Fv fragment.

In still other embodiments, the invention provides an isolated human antibody, or an  
15 antigen-binding portions thereof, having D2E7-related VL and VH CDR3 domains, for example, antibodies, or antigen-binding portions thereof, with a light chain variable region (LCVR) having a CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO:  
20 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25 and SEQ ID NO: 26 or with a heavy chain variable region (HCVR) having a CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34 and SEQ ID NO: 35.

25 In yet another embodiment, the invention provides a human antibody, or antigen-binding portion thereof, that neutralizes the activity of human TNF $\alpha$ , chimpanzee TNF $\alpha$  and at least one additional primate TNF $\alpha$  selected from the group consisting of baboon TNF $\alpha$ , marmoset TNF $\alpha$ , cynomolgus TNF $\alpha$  and rhesus TNF $\alpha$ . Preferably, the antibody, or antigen-binding portion thereof, neutralizes the human, chimpanzee and/or additional primate TNF $\alpha$   
30 in a standard *in vitro* L929 assay with an IC<sub>50</sub> of  $1 \times 10^{-8}$  M or less, more preferably  $1 \times 10^{-9}$  M or less, even more preferably  $1 \times 10^{-10}$  M or less and still more preferably  $1 \times 10^{-11}$  M or less. In one subembodiment, the antibody also neutralizes the activity of mouse TNF $\alpha$ , preferably in a standard *in vitro* L929 assay with an IC<sub>50</sub> of  $1 \times 10^{-6}$  M or less, more preferably  $1 \times 10^{-7}$  M or less and even more preferably  $1 \times 10^{-8}$  M or less. In another  
35 subembodiment, the antibody also neutralizes the activity of pig TNF $\alpha$ , preferably with an IC<sub>50</sub> of  $1 \times 10^{-6}$  M or less, more preferably  $1 \times 10^{-7}$  M or less and even more preferably  $1 \times 10^{-8}$  M or less.. In another preferred subembodiment, the antibody is a recombinant antibody.

An antibody or antibody portion of the invention can be derivatized or linked to another functional molecule (e.g., another peptide or protein). Accordingly, the antibodies and antibody portions of the invention are intended to include derivatized and otherwise modified forms of the human anti-hTNF $\alpha$  antibodies described herein, including immunoadhesion molecules. For example, an antibody or antibody portion of the invention can be functionally linked (by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody (e.g., a bispecific antibody or a diabody), a detectable agent, a cytotoxic agent, a pharmaceutical agent, and/or a protein or peptide that can mediate association of the antibody or antibody portion with another molecule (such as a streptavidin core region or a polyhistidine tag).

One type of derivatized antibody is produced by crosslinking two or more antibodies (of the same type or of different types, e.g., to create bispecific antibodies). Suitable crosslinkers include those that are heterobifunctional, having two distinctly reactive groups separated by an appropriate spacer (e.g., m-maleimidobenzoyl-N-hydroxysuccinimide ester) or homobifunctional (e.g., disuccinimidyl suberate). Such linkers are available from Pierce Chemical Company, Rockford, IL.

Useful detectable agents with which an antibody or antibody portion of the invention may be derivatized include fluorescent compounds. Exemplary fluorescent detectable agents include fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-1-naphthalenesulfonyl chloride, phycoerythrin and the like. An antibody may also be derivatized with detectable enzymes, such as alkaline phosphatase, horseradish peroxidase, glucose oxidase and the like. When an antibody is derivatized with a detectable enzyme, it is detected by adding additional reagents that the enzyme uses to produce a detectable reaction product. For example, when the detectable agent horseradish peroxidase is present, the addition of hydrogen peroxide and diaminobenzidine leads to a colored reaction product, which is detectable. An antibody may also be derivatized with biotin, and detected through indirect measurement of avidin or streptavidin binding.

## II. Expression of Antibodies

An antibody, or antibody portion, of the invention can be prepared by recombinant expression of immunoglobulin light and heavy chain genes in a host cell. To express an antibody recombinantly, a host cell is transfected with one or more recombinant expression vectors carrying DNA fragments encoding the immunoglobulin light and heavy chains of the antibody such that the light and heavy chains are expressed in the host cell and, preferably, secreted into the medium in which the host cells are cultured, from which medium the antibodies can be recovered. Standard recombinant DNA methodologies are used to obtain antibody heavy and light chain genes, incorporate these genes into recombinant expression vectors and introduce the vectors into host cells, such as those described in Sambrook, Fritsch and Maniatis (eds), *Molecular Cloning: A Laboratory Manual, Second Edition*, Cold Spring

Harbor, N.Y., (1989), Ausubel, F.M. *et al.* (eds.) *Current Protocols in Molecular Biology*, Greene Publishing Associates, (1989) and in U.S. Patent No. 4,816,397 by Boss *et al.*

To express D2E7 or a D2E7-related antibody, DNA fragments encoding the light and heavy chain variable regions are first obtained. These DNAs can be obtained by amplification and modification of germline light and heavy chain variable sequences using the polymerase chain reaction (PCR). Germline DNA sequences for human heavy and light chain variable region genes are known in the art (see *e.g.*, the "Vbase" human germline sequence database; see also Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest*, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242; Tomlinson, I.M., *et al.* (1992) "The Repertoire of Human Germline V<sub>H</sub> Sequences Reveals about Fifty Groups of V<sub>H</sub> Segments with Different Hypervariable Loops" *J. Mol. Biol.* 227:776-798; and Cox, J.P.L. *et al.* (1994) "A Directory of Human Germ-line V<sub>K</sub> Segments Reveals a Strong Bias in their Usage" *Eur. J. Immunol.* 24:827-836; the contents of each of which are expressly incorporated herein by reference). To obtain a DNA fragment encoding the heavy chain variable region of D2E7, or a D2E7-related antibody, a member of the V<sub>H</sub>3 family of human germline VH genes is amplified by standard PCR. Most preferably, the DP-31 VH germline sequence is amplified. To obtain a DNA fragment encoding the light chain variable region of D2E7, or a D2E7-related antibody, a member of the V<sub>K</sub>I family of human germline VL genes is amplified by standard PCR. Most preferably, the A20 VL germline sequence is amplified. PCR primers suitable for use in amplifying the DP-31 germline VH and A20 germline VL sequences can be designed based on the nucleotide sequences disclosed in the references cited *supra*, using standard methods.

Once the germline VH and VL fragments are obtained, these sequences can be mutated to encode the D2E7 or D2E7-related amino acid sequences disclosed herein. The amino acid sequences encoded by the germline VH and VL DNA sequences are first compared to the D2E7 or D2E7-related VH and VL amino acid sequences to identify amino acid residues in the D2E7 or D2E7-related sequence that differ from germline. Then, the appropriate nucleotides of the germline DNA sequences are mutated such that the mutated germline sequence encodes the D2E7 or D2E7-related amino acid sequence, using the genetic code to determine which nucleotide changes should be made. Mutagenesis of the germline sequences is carried out by standard methods, such as PCR-mediated mutagenesis (in which the mutated nucleotides are incorporated into the PCR primers such that the PCR product contains the mutations) or site-directed mutagenesis.

Once DNA fragments encoding D2E7 or D2E7-related VH and VL segments are obtained (by amplification and mutagenesis of germline VH and VL genes, as described above), these DNA fragments can be further manipulated by standard recombinant DNA techniques, for example to convert the variable region genes to full-length antibody chain genes, to Fab fragment genes or to a scFv gene. In these manipulations, a VL- or VH-encoding DNA fragment is operatively linked to another DNA fragment encoding another

protein, such as an antibody constant region or a flexible linker. The term "operatively linked", as used in this context, is intended to mean that the two DNA fragments are joined such that the amino acid sequences encoded by the two DNA fragments remain in-frame.

The isolated DNA encoding the VH region can be converted to a full-length heavy chain gene by operatively linking the VH-encoding DNA to another DNA molecule encoding heavy chain constant regions (CH1, CH2 and CH3). The sequences of human heavy chain constant region genes are known in the art (see *e.g.*, Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The heavy chain constant region can be an IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM or IgD constant region, but most preferably is an IgG1 or IgG4 constant region. For a Fab fragment heavy chain gene, the VH-encoding DNA can be operatively linked to another DNA molecule encoding only the heavy chain CH1 constant region.

The isolated DNA encoding the VL region can be converted to a full-length light chain gene (as well as a Fab light chain gene) by operatively linking the VL-encoding DNA to another DNA molecule encoding the light chain constant region, CL. The sequences of human light chain constant region genes are known in the art (see *e.g.*, Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The light chain constant region can be a kappa or lambda constant region, but most preferably is a kappa constant region.

To create a scFv gene, the VH- and VL-encoding DNA fragments are operatively linked to another fragment encoding a flexible linker, *e.g.*, encoding the amino acid sequence (Gly<sub>4</sub>-Ser)<sub>3</sub>, such that the VH and VL sequences can be expressed as a contiguous single-chain protein, with the VL and VH regions joined by the flexible linker (see *e.g.*, Bird *et al.* (1988) *Science* 242:423-426; Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883; McCafferty *et al.*, *Nature* (1990) 348:552-554).

To express the antibodies, or antibody portions of the invention, DNAs encoding partial or full-length light and heavy chains, obtained as described above, are inserted into expression vectors such that the genes are operatively linked to transcriptional and translational control sequences. In this context, the term "operatively linked" is intended to mean that an antibody gene is ligated into a vector such that transcriptional and translational control sequences within the vector serve their intended function of regulating the transcription and translation of the antibody gene. The expression vector and expression control sequences are chosen to be compatible with the expression host cell used. The antibody light chain gene and the antibody heavy chain gene can be inserted into separate vector or, more typically, both genes are inserted into the same expression vector. The

antibody genes are inserted into the expression vector by standard methods (e.g., ligation of complementary restriction sites on the antibody gene fragment and vector, or blunt end ligation if no restriction sites are present). Prior to insertion of the D2E7 or D2E7-related light or heavy chain sequences, the expression vector may already carry antibody constant region sequences. For example, one approach to converting the D2E7 or D2E7-related VH and VL sequences to full-length antibody genes is to insert them into expression vectors already encoding heavy chain constant and light chain constant regions, respectively, such that the VH segment is operatively linked to the CH segment(s) within the vector and the VL segment is operatively linked to the CL segment within the vector. Additionally or alternatively, the recombinant expression vector can encode a signal peptide that facilitates secretion of the antibody chain from a host cell. The antibody chain gene can be cloned into the vector such that the signal peptide is linked in-frame to the amino terminus of the antibody chain gene. The signal peptide can be an immunoglobulin signal peptide or a heterologous signal peptide (i.e., a signal peptide from a non-immunoglobulin protein).

In addition to the antibody chain genes, the recombinant expression vectors of the invention carry regulatory sequences that control the expression of the antibody chain genes in a host cell. The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (e.g., polyadenylation signals) that control the transcription or translation of the antibody chain genes. Such regulatory sequences are described, for example, in Goeddel; *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). It will be appreciated by those skilled in the art that the design of the expression vector, including the selection of regulatory sequences may depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. Preferred regulatory sequences for mammalian host cell expression include viral elements that direct high levels of protein expression in mammalian cells, such as promoters and/or enhancers derived from cytomegalovirus (CMV) (such as the CMV promoter/enhancer), Simian Virus 40 (SV40) (such as the SV40 promoter/enhancer), adenovirus, (e.g., the adenovirus major late promoter (AdMLP)) and polyoma. For further description of viral regulatory elements, and sequences thereof, see e.g., U.S. Patent No. 5,168,062 by Stinski, U.S. Patent No. 4,510,245 by Bell *et al.* and U.S. Patent No. 4,968,615 by Schaffner *et al.*

In addition to the antibody chain genes and regulatory sequences, the recombinant expression vectors of the invention may carry additional sequences, such as sequences that regulate replication of the vector in host cells (e.g., origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see e.g., U.S. Patents Nos. 4,399,216, 4,634,665 and 5,179,017, all by Axel *et al.*). For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Preferred selectable marker genes include the dihydrofolate reductase

(DHFR) gene (for use in dhfr<sup>-</sup> host cells with methotrexate selection/amplification) and the *neo* gene (for G418 selection).

For expression of the light and heavy chains, the expression vector(s) encoding the heavy and light chains is transfected into a host cell by standard techniques. The various forms of the term "transfection" are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, *e.g.*, electroporation, calcium-phosphate precipitation, DEAE-dextran transfection and the like. Although it is theoretically possible to express the antibodies of the invention in either prokaryotic or eukaryotic host cells, expression of antibodies in eukaryotic cells, and most preferably mammalian host cells, is the most preferred because such eukaryotic cells, and in particular mammalian cells, are more likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active antibody. Prokaryotic expression of antibody genes has been reported to be ineffective for production of high yields of active antibody (Boss, M.A. and Wood, C. R. (1985) *Immunology Today* 6:12-13).

Preferred mammalian host cells for expressing the recombinant antibodies of the invention include Chinese Hamster Ovary (CHO cells) (including dhfr<sup>-</sup> CHO cells, described in Urlaub and Chasin, (1980) *Proc. Natl. Acad. Sci. USA* 77:4216-4220, used with a DHFR selectable marker, *e.g.*, as described in R.J. Kaufman and P.A. Sharp (1982) *Mol. Biol.* 159:601-621), NS0 myeloma cells, COS cells and SP2 cells. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods.

Host cells can also be used to produce portions of intact antibodies, such as Fab fragments or scFv molecules. It will be understood that variations on the above procedure are within the scope of the present invention. For example, it may be desirable to transfect a host cell with DNA encoding either the light chain or the heavy chain (but not both) of an antibody of this invention. Recombinant DNA technology may also be used to remove some or all of the DNA encoding either or both of the light and heavy chains that is not necessary for binding to hTNF $\alpha$ . The molecules expressed from such truncated DNA molecules are also encompassed by the antibodies of the invention. In addition, bifunctional antibodies may be produced in which one heavy and one light chain are an antibody of the invention and the other heavy and light chain are specific for an antigen other than hTNF $\alpha$  by crosslinking an antibody of the invention to a second antibody by standard chemical crosslinking methods.

In a preferred system for recombinant expression of an antibody, or antigen-binding portion thereof, of the invention, a recombinant expression vector encoding both the antibody heavy chain and the antibody light chain is introduced into dhfr<sup>-</sup> CHO cells by calcium phosphate-mediated transfection. Within the recombinant expression vector, the antibody

heavy and light chain genes are each operatively linked to CMV enhancer/AdMLP promoter regulatory elements to drive high levels of transcription of the genes. The recombinant expression vector also carries a DHFR gene, which allows for selection of CHO cells that have been transfected with the vector using methotrexate selection/amplification. The selected transformant host cells are culture to allow for expression of the antibody heavy and light chains and intact antibody is recovered from the culture medium. Standard molecular biology techniques are used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture the host cells and recover the antibody from the culture medium.

In view of the foregoing, another aspect of the invention pertains to nucleic acid, vector and host cell compositions that can be used for recombinant expression of the antibodies and antibody portions of the invention. The nucleotide sequence encoding the D2E7 light chain variable region is shown in Figure 7 and SEQ ID NO: 36. The CDR1 domain of the LCVR encompasses nucleotides 70-102, the CDR2 domain encompasses nucleotides 148-168 and the CDR3 domain encompasses nucleotides 265-291. The nucleotide sequence encoding the D2E7 heavy chain variable region is shown in Figure 8 and SEQ ID NO: 37. The CDR1 domain of the HCVR encompasses nucleotides 91-105, the CDR2 domain encompasses nucleotides 148-198 and the CDR3 domain encompasses nucleotides 295-330. It will be appreciated by the skilled artisan that nucleotide sequences encoding D2E7-related antibodies, or portions thereof (*e.g.*, a CDR domain, such as a CDR3 domain), can be derived from the nucleotide sequences encoding the D2E7 LCVR and HCVR using the genetic code and standard molecular biology techniques.

In one embodiment, the invention provides an isolated nucleic acid encoding a light chain CDR3 domain comprising the amino acid sequence of SEQ ID NO: 3 (*i.e.*, the D2E7 VL CDR3), or modified from SEQ ID NO: 3 by a single alanine substitution at position 1, 4, 5, 7 or 8 or by one to five conservative amino acid substitutions at positions 1, 3, 4, 6, 7, 8 and/or 9. This nucleic acid can encode only the CDR3 region or, more preferably, encodes an entire antibody light chain variable region (LCVR). For example, the nucleic acid can encode an LCVR having a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 5 (*i.e.*, the D2E7 VL CDR2) and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 7 (*i.e.*, the D2E7 VL CDR1).

In another embodiment, the invention provides an isolated nucleic acid encoding a heavy chain CDR3 domain comprising the amino acid sequence of SEQ ID NO: 4 (*i.e.*, the D2E7 VH CDR3), or modified from SEQ ID NO: 4 by a single alanine substitution at position 2, 3, 4, 5, 6, 8, 9, 10 or 11 or by one to five conservative amino acid substitutions at positions 2, 3, 4, 5, 6, 8, 9, 10, 11 and/or 12. This nucleic acid can encode only the CDR3 region or, more preferably, encodes an entire antibody heavy chain variable region (HCVR). For example, the nucleic acid can encode a HCVR having a CDR2 domain comprising the

amino acid sequence of SEQ ID NO: 6 (*i.e.*, the D2E7 VH CDR2) and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 8 (*i.e.*, the D2E7 VH CDR1).

In yet another embodiment, the invention provides isolated nucleic acids encoding a D2E7-related CDR3 domain, *e.g.*, comprising an amino acid sequence selected from the group consisting of: SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34 and SEQ ID NO: 35.

In still another embodiment, the invention provides an isolated nucleic acid encoding an antibody light chain variable region comprising the amino acid sequence of SEQ ID NO: 1 (*i.e.*, the D2E7 LCVR). Preferably this nucleic acid comprises the nucleotide sequence of SEQ ID NO: 36, although the skilled artisan will appreciate that due to the degeneracy of the genetic code, other nucleotide sequences can encode the amino acid sequence of SEQ ID NO: 1. The nucleic acid can encode only the LCVR or can also encode an antibody light chain constant region, operatively linked to the LCVR. In one embodiment, this nucleic acid is in a recombinant expression vector.

In still another embodiment, the invention provides an isolated nucleic acid encoding an antibody heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 2 (*i.e.*, the D2E7 HCVR). Preferably this nucleic acid comprises the nucleotide sequence of SEQ ID NO: 37, although the skilled artisan will appreciate that due to the degeneracy of the genetic code, other nucleotide sequences can encode the amino acid sequence of SEQ ID NO: 2. The nucleic acid can encode only the HCVR or can also encode a heavy chain constant region, operatively linked to the HCVR. For example, the nucleic acid can comprise an IgG1 or IgG4 constant region. In one embodiment, this nucleic acid is in a recombinant expression vector.

The invention also provides recombinant expression vectors encoding both an antibody heavy chain and an antibody light chain. For example, in one embodiment, the invention provides a recombinant expression vector encoding:

- a) an antibody light chain having a variable region comprising the amino acid sequence of SEQ ID NO: 1 (*i.e.*, the D2E7 LCVR); and
- b) an antibody heavy chain having a variable region comprising the amino acid sequence of SEQ ID NO: 2 (*i.e.*, the D2E7 HCVR).

The invention also provides host cells into which one or more of the recombinant expression vectors of the invention have been introduced. Preferably, the host cell is a mammalian host cell, more preferably the host cell is a CHO cell, an NS0 cell or a COS cell. Still further the invention provides a method of synthesizing a recombinant human antibody of the invention by culturing a host cell of the invention in a suitable culture medium until a



recombinant human antibody of the invention is synthesized. The method can further comprise isolating the recombinant human antibody from the culture medium.

### III. Selection of Recombinant Human Antibodies

Recombinant human antibodies of the invention in addition to the D2E7 or D2E7-related antibodies disclosed herein can be isolated by screening of a recombinant combinatorial antibody library, preferably a scFv phage display library, prepared using human VL and VH cDNAs prepared from mRNA derived from human lymphocytes. Methodologies for preparing and screening such libraries are known in the art. In addition to commercially available kits for generating phage display libraries (e.g., the Pharmacia *Recombinant Phage Antibody System*, catalog no. 27-9400-01; and the Stratagene *SurfZAP*<sup>TM</sup> phage display kit, catalog no. 240612), examples of methods and reagents particularly amenable for use in generating and screening antibody display libraries can be found in, for example, Ladner *et al.* U.S. Patent No. 5,223,409; Kang *et al.* PCT Publication No. WO 92/18619; Dower *et al.* PCT Publication No. WO 91/17271; Winter *et al.* PCT Publication No. WO 92/20791; Markland *et al.* PCT Publication No. WO 92/15679; Breitling *et al.* PCT Publication No. WO 93/01288; McCafferty *et al.* PCT Publication No. WO 92/01047; Garrard *et al.* PCT Publication No. WO 92/09690; Fuchs *et al.* (1991) *Bio/Technology* 2:1370-1372; Hay *et al.* (1992) *Hum Antibod Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281; McCafferty *et al.*, *Nature* (1990) 348:552-554; Griffiths *et al.* (1993) *EMBO J* 12:725-734; Hawkins *et al.* (1992) *J Mol Biol* 226:889-896; Clackson *et al.* (1991) *Nature* 352:624-628; Gram *et al.* (1992) *PNAS* 89:3576-3580; Garrard *et al.* (1991) *Bio/Technology* 2:1373-1377; Hoogenboom *et al.* (1991) *Nuc Acid Res* 19:4133-4137; and Barbas *et al.* (1991) *PNAS* 88:7978-7982.

In a preferred embodiment, to isolate human antibodies with high affinity and a low off rate constant for hTNF $\alpha$ , a murine anti-hTNF $\alpha$  antibody having high affinity and a low off rate constant for hTNF $\alpha$  (e.g., MAK 195, the hybridoma for which has deposit number ECACC 87 050801) is first used to select human heavy and light chain sequences having similar binding activity toward hTNF $\alpha$ , using the epitope imprinting methods described in Hoogenboom *et al.*, PCT Publication No. WO 93/06213. The antibody libraries used in this method are preferably scFv libraries prepared and screened as described in McCafferty *et al.*, PCT Publication No. WO 92/01047, McCafferty *et al.*, *Nature* (1990) 348:552-554; and Griffiths *et al.*, (1993) *EMBO J* 12:725-734. The scFv antibody libraries preferably are screened using recombinant human TNF $\alpha$  as the antigen.

Once initial human VL and VH segments are selected, "mix and match" experiments, in which different pairs of the initially selected VL and VH segments are screened for hTNF $\alpha$  binding, are performed to select preferred VL/VH pair combinations. Additionally, to further improve the affinity and/or lower the off rate constant for hTNF $\alpha$  binding, the VL and VH segments of the preferred VL/VH pair(s) can be randomly mutated, preferably within the

CDR3 region of VH and/or VL, in a process analogous to the *in vivo* somatic mutation process responsible for affinity maturation of antibodies during a natural immune response. This *in vitro* affinity maturation can be accomplished by amplifying VH and VL regions using PCR primers complimentary to the VH CDR3 or VL CDR3, respectively, which  
5 primers have been "spiked" with a random mixture of the four nucleotide bases at certain positions such that the resultant PCR products encode VH and VL segments into which random mutations have been introduced into the VH and/or VL CDR3 regions. These randomly mutated VH and VL segments can be rescreened for binding to hTNF $\alpha$  and sequences that exhibit high affinity and a low off rate for hTNF $\alpha$  binding can be selected.

10 Following screening and isolation of an anti-hTNF $\alpha$  antibody of the invention from a recombinant immunoglobulin display library, nucleic acid encoding the selected antibody can be recovered from the display package (*e.g.*, from the phage genome) and subcloned into other expression vectors by standard recombinant DNA techniques. If desired, the nucleic acid can be further manipulated to create other antibody forms of the invention (*e.g.*, linked to  
15 nucleic acid encoding additional immunoglobulin domains, such as additional constant regions). To express a recombinant human antibody isolated by screening of a combinatorial library, the DNA encoding the antibody is cloned into a recombinant expression vector and introduced into a mammalian host cells, as described in further detail in Section II above.

#### 20 IV. Pharmaceutical Compositions and Pharmaceutical Administration

The antibodies and antibody-portions of the invention can be incorporated into pharmaceutical compositions suitable for administration to a subject. Typically, the pharmaceutical composition comprises an antibody or antibody portion of the invention and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier"  
25 includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Examples of pharmaceutically acceptable carriers include one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In many cases, it will be preferable to include isotonic agents, for example, sugars,  
30 polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Pharmaceutically acceptable carriers may further comprise minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the antibody or antibody portion.

The compositions of this invention may be in a variety of forms. These include, for  
35 example, liquid, semi-solid and solid dosage forms, such as liquid solutions (*e.g.*, injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The preferred form depends on the intended mode of administration and therapeutic application. Typical preferred compositions are in the form of injectable or infusible solutions, such as compositions similar to those used for passive immunization of

humans with other antibodies. The preferred mode of administration is parenteral (*e.g.*, intravenous, subcutaneous, intraperitoneal, intramuscular). In a preferred embodiment, the antibody is administered by intravenous infusion or injection. In another preferred embodiment, the antibody is administered by intramuscular or subcutaneous injection.

Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the active compound (*i.e.*, antibody or antibody portion) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

The antibodies and antibody-portions of the present invention can be administered by a variety of methods known in the art, although for many therapeutic applications, the preferred route/mode of administration is intravenous injection or infusion. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. In certain embodiments, the active compound may be prepared with a carrier that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. See, *e.g.*, *Sustained and Controlled Release Drug Delivery Systems*, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

In certain embodiments, an antibody or antibody portion of the invention may be orally administered, for example, with an inert diluent or an assimilable edible carrier. The compound (and other ingredients, if desired) may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the subject's diet. For oral therapeutic administration, the compounds may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. To administer a compound of the invention by other than parenteral

administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation.

Supplementary active compounds can also be incorporated into the compositions. In certain embodiments, an antibody or antibody portion of the invention is coformulated with and/or coadministered with one or more additional therapeutic agents. For example, an anti-hTNF $\alpha$  antibody or antibody portion of the invention may be coformulated and/or coadministered with one or more additional antibodies that bind other targets (*e.g.*, antibodies that bind other cytokines or that bind cell surface molecules), one or more cytokines, soluble TNF $\alpha$  receptor (see *e.g.*, PCT Publication No. WO 94/06476) and/or one or more chemical agents that inhibit hTNF $\alpha$  production or activity (such as cyclohexane-ylidene derivatives as described in PCT Publication No. WO 93/19751). Furthermore, one or more antibodies of the invention may be used in combination with two or more of the foregoing therapeutic agents. Such combination therapies may advantageously utilize lower dosages of the administered therapeutic agents, thus avoiding possible toxicities or complications associated with the various monotherapies. The use of the antibodies, or antibody portions, of the invention in combination with other therapeutic agents is discussed further in subsection IV.

The pharmaceutical compositions of the invention may include a "therapeutically effective amount" or a "prophylactically effective amount" of an antibody or antibody portion of the invention. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the antibody or antibody portion may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody or antibody portion to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody or antibody portion are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

Dosage regimens may be adjusted to provide the optimum desired response (*e.g.*, a therapeutic or prophylactic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent

on (a) the unique characteristics of the active compound and the particular therapeutic or prophylactic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

5 An exemplary, non-limiting range for a therapeutically or prophylactically effective amount of an antibody or antibody portion of the invention is 0.1-20 mg/kg, more preferably 1-10 mg/kg. It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of  
10 the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

#### IV. Uses of the Antibodies of the Invention

Given their ability to bind to hTNF $\alpha$ , the anti-hTNF $\alpha$  antibodies, or portions thereof,  
15 of the invention can be used to detect hTNF $\alpha$  (e.g., in a biological sample, such as serum or plasma), using a conventional immunoassay, such as an enzyme linked immunosorbent assays (ELISA), an radioimmunoassay (RIA) or tissue immunohistochemistry. The invention provides a method for detecting hTNF $\alpha$  in a biological sample comprising contacting a biological sample with an antibody, or antibody portion, of the invention and  
20 detecting either the antibody (or antibody portion) bound to hTNF $\alpha$  or unbound antibody (or antibody portion), to thereby detect hTNF $\alpha$  in the biological sample. The antibody is directly or indirectly labeled with a detectable substance to facilitate detection of the bound or unbound antibody. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials and radioactive materials. Examples of  
25 suitable enzymes include horseradish peroxidase, alkaline phosphatase,  $\beta$ -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes  
30 luminol; and examples of suitable radioactive material include  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{35}\text{S}$  or  $^3\text{H}$ .

Alternative to labeling the antibody, hTNF $\alpha$  can be assayed in biological fluids by a competition immunoassay utilizing rhTNF $\alpha$  standards labeled with a detectable substance and an unlabeled anti-hTNF $\alpha$  antibody. In this assay, the biological sample, the labeled  
35 rhTNF $\alpha$  standards and the anti-hTNF $\alpha$  antibody are combined and the amount of labeled rhTNF $\alpha$  standard bound to the unlabeled antibody is determined. The amount of hTNF $\alpha$  in the biological sample is inversely proportional to the amount of labeled rhTNF $\alpha$  standard bound to the anti-hTNF $\alpha$  antibody.

A D2E7 antibody of the invention can also be used to detect TNF $\alpha$ s from species other than humans, in particular TNF $\alpha$ s from primates (e.g., chimpanzee, baboon, marmoset,

cynomolgus and rhesus), pig and mouse, since D2E7 can bind to each of these TNF $\alpha$ s (discussed further in Example 4, subsection E).

5 The antibodies and antibody portions of the invention are capable of neutralizing hTNF $\alpha$  activity both *in vitro* and *in vivo* (see Example 4). Moreover, at least some of the antibodies of the invention, such as D2E7, can neutralize TNF $\alpha$  activity from other species. Accordingly, the antibodies and antibody portions of the invention can be used to inhibit TNF $\alpha$  activity, *e.g.*, in a cell culture containing hTNF $\alpha$ , in human subjects or in other mammalian subjects having TNF $\alpha$ s with which an antibody of the invention cross-reacts (*e.g.* chimpanzee, baboon, marmoset, cynomolgus and rhesus, pig or mouse). In one  
10 embodiment, the invention provides a method for inhibiting TNF $\alpha$  activity comprising contacting TNF $\alpha$  with an antibody or antibody portion of the invention such that TNF $\alpha$  activity is inhibited. Preferably, the TNF $\alpha$  is human TNF $\alpha$ . For example, in a cell culture containing, or suspected of containing hTNF $\alpha$ , an antibody or antibody portion of the invention can be added to the culture medium to inhibit hTNF $\alpha$  activity in the culture.

15 In another embodiment, the invention provides a method for inhibiting TNF $\alpha$  activity in a subject suffering from a disorder in which TNF $\alpha$  activity is detrimental, comprising administering to the subject an antibody or antibody portion of the invention such that TNF $\alpha$  activity in the subject is inhibited. Preferably, the TNF $\alpha$  is human TNF $\alpha$  and the subject is a human subject. Alternatively, the subject can be a mammal expressing a TNF $\alpha$  with which  
20 an antibody of the invention cross-reacts. Still further the subject can be a mammal into which has been introduced hTNF $\alpha$  (*e.g.*, by administration of hTNF $\alpha$  or by expression of an hTNF $\alpha$  transgene). An antibody of the invention can be administered to a human subject for therapeutic purposes (discussed further below). Moreover, an antibody of the invention can be administered to a non-human mammal expressing a TNF $\alpha$  with which the antibody cross-  
25 reacts (*e.g.*, a primate, pig or mouse) for veterinary purposes or as an animal model of human disease. Regarding the latter, such animal models may be useful for evaluating the therapeutic efficacy of antibodies of the invention (*e.g.*, testing of dosages and time courses of administration).

As used herein, the term "a disorder in which TNF $\alpha$  activity is detrimental" is  
30 intended to include diseases and other disorders in which the presence of TNF $\alpha$  in a subject suffering from the disorder has been shown to be or is suspected of being either responsible for the pathophysiology of the disorder or a factor that contributes to a worsening of the disorder. Accordingly, a disorder in which TNF $\alpha$  activity is detrimental is a disorder in which inhibition of TNF $\alpha$  activity is expected to alleviate the symptoms and/or progression  
35 of the disorder. Such disorders may be evidenced, for example, by an increase in the concentration of TNF $\alpha$  in a biological fluid of a subject suffering from the disorder (*e.g.*, an increase in the concentration of TNF $\alpha$  in serum, plasma, synovial fluid, *etc.* of the subject), which can be detected, for example, using an anti-TNF $\alpha$  antibody as described above. There are numerous examples of disorders in which TNF $\alpha$  activity is detrimental. The use of the

antibodies and antibody portions of the invention in the treatment of specific disorders is discussed further below:

#### A. Sepsis

- 5 Tumor necrosis factor has an established role in the pathophysiology of sepsis, with biological effects that include hypotension, myocardial suppression, vascular leakage syndrome, organ necrosis, stimulation of the release of toxic secondary mediators and activation of the clotting cascade (see *e.g.*, Tracey, K.J. and Cerami, A. (1994) *Annu. Rev. Med.* 45:491-503; Russell, D and Thompson, R.C. (1993) *Curr. Opin. Biotech.* 4:714-721).
- 10 Accordingly, the human antibodies, and antibody portions, of the invention can be used to treat sepsis in any of its clinical settings, including septic shock, endotoxic shock, gram negative sepsis and toxic shock syndrome.

Furthermore, to treat sepsis, an anti-hTNF $\alpha$  antibody, or antibody portion, of the invention can be coadministered with one or more additional therapeutic agents that may

15 further alleviate sepsis, such as an interleukin-1 inhibitor (such as those described in PCT Publication Nos. WO 92/16221 and WO 92/17583), the cytokine interleukin-6 (see *e.g.*, PCT Publication No. WO 93/11793) or an antagonist of platelet activating factor (see *e.g.*, European Patent Application Publication No. EP 374 510).

Additionally, in a preferred embodiment, an anti-TNF $\alpha$  antibody or antibody portion

20 of the invention is administered to a human subject within a subgroup of sepsis patients having a serum or plasma concentration of IL-6 above 500 pg/ml, and more preferably 1000 pg/ml, at the time of treatment (see PCT Publication No. WO 95/20978 by Daum, L., *et al.*).

#### B. Autoimmune Diseases

- 25 Tumor necrosis factor has been implicated in playing a role in the pathophysiology of a variety of autoimmune diseases. For example, TNF $\alpha$  has been implicated in activating tissue inflammation and causing joint destruction in rheumatoid arthritis (see *e.g.*, Tracey and Cerami, *supra*; Arend, W.P. and Dayer, J-M. (1995) *Arth. Rheum.* 38:151-160; Fava, R.A., *et al.* (1993) *Clin. Exp. Immunol.* 94:261-266). TNF $\alpha$  also has been implicated in promoting
- 30 the death of islet cells and in mediating insulin resistance in diabetes (see *e.g.*, Tracey and Cerami, *supra*; PCT Publication No. WO 94/08609). TNF $\alpha$  also has been implicated in mediating cytotoxicity to oligodendrocytes and induction of inflammatory plaques in multiple sclerosis (see *e.g.*, Tracey and Cerami, *supra*). Chimeric and humanized murine anti-hTNF $\alpha$  antibodies have undergone clinical testing for treatment of rheumatoid arthritis
- 35 (see *e.g.*, Elliott, M.J., *et al.* (1994) *Lancet* 344:1125-1127; Elliott, M.J., *et al.* (1994) *Lancet* 344:1105-1110; Rankin, E.C., *et al.* (1995) *Br. J. Rheumatol.* 34:334-342).

The human antibodies, and antibody portions of the invention can be used to treat autoimmune diseases, in particular those associated with inflammation, including rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis and gouty arthritis, allergy, multiple sclerosis,

autoimmune diabetes, autoimmune uveitis and nephrotic syndrome. Typically, the antibody, or antibody portion, is administered systemically, although for certain disorders, local administration of the antibody or antibody portion at a site of inflammation may be beneficial (e.g., local administration in the joints in rheumatoid arthritis or topical application to diabetic ulcers, alone or in combination with a cyclohexane-ylidene derivative as described in PCT Publication No. WO 93/19751).

### C. Infectious Diseases

Tumor necrosis factor has been implicated in mediating biological effects observed in a variety of infectious diseases. For example, TNF $\alpha$  has been implicated in mediating brain inflammation and capillary thrombosis and infarction in malaria (see e.g., Tracey and Cerami, *supra*). TNF $\alpha$  also has been implicated in mediating brain inflammation, inducing breakdown of the blood-brain barrier, triggering septic shock syndrome and activating venous infarction in meningitis (see e.g., Tracey and Cerami, *supra*). TNF $\alpha$  also has been implicated in inducing cachexia, stimulating viral proliferation and mediating central nervous system injury in acquired immune deficiency syndrome (AIDS) (see e.g., Tracey and Cerami, *supra*). Accordingly, the antibodies, and antibody portions, of the invention, can be used in the treatment of infectious diseases, including bacterial meningitis (see e.g., European Patent Application Publication No. EP 585 705), cerebral malaria, AIDS and AIDS-related complex (ARC) (see e.g., European Patent Application Publication No. EP 230 574), as well as cytomegalovirus infection secondary to transplantation (see e.g., Fietze, E., *et al.* (1994) *Transplantation* 58:675-680). The antibodies, and antibody portions, of the invention, also can be used to alleviate symptoms associated with infectious diseases, including fever and myalgias due to infection (such as influenza) and cachexia secondary to infection (e.g., secondary to AIDS or ARC).

### D. Transplantation

Tumor necrosis factor has been implicated as a key mediator of allograft rejection and graft versus host disease (GVHD) and in mediating an adverse reaction that has been observed when the rat antibody OKT3, directed against the T cell receptor CD3 complex, is used to inhibit rejection of renal transplants (see e.g., Tracey and Cerami, *supra*; Eason, J.D., *et al.* (1995) *Transplantation* 59:300-305; Suthanthiran, M. and Strom, T.B. (1994) *New Engl. J. Med.* 331:365-375). Accordingly, the antibodies, and antibody portions, of the invention, can be used to inhibit transplant rejection, including rejections of allografts and xenografts and to inhibit GVHD. Although the antibody or antibody portion may be used alone, more preferably it is used in combination with one or more other agents that inhibit the immune response against the allograft or inhibit GVHD. For example, in one embodiment, an antibody or antibody portion of the invention is used in combination with OKT3 to inhibit OKT3-induced reactions. In another embodiment, an antibody or antibody portion of the



invention is used in combination with one or more antibodies directed at other targets involved in regulating immune responses, such as the cell surface molecules CD25 (interleukin-2 receptor- $\alpha$ ), CD11a (LFA-1), CD54 (ICAM-1), CD4, CD45, CD28/CTLA4, CD80 (B7-1) and/or CD86 (B7-2). In yet another embodiment, an antibody or antibody  
5 portion of the invention is used in combination with one or more general immunosuppressive agents, such as cyclosporin A or FK506.

#### E. Malignancy

Tumor necrosis factor has been implicated in inducing cachexia, stimulating tumor  
10 growth, enhancing metastatic potential and mediating cytotoxicity in malignancies (see *e.g.*, Tracey and Cerami, *supra*). Accordingly, the antibodies, and antibody portions, of the invention, can be used in the treatment of malignancies, to inhibit tumor growth or metastasis and/or to alleviate cachexia secondary to malignancy. The antibody, or antibody portion, may be administered systemically or locally to the tumor site.

#### F. Pulmonary Disorders

Tumor necrosis factor has been implicated in the pathophysiology of adult respiratory distress syndrome, including stimulating leukocyte-endothelial activation, directing cytotoxicity to pneumocytes and inducing vascular leakage syndrome (see *e.g.*, Tracey and  
20 Cerami, *supra*). Accordingly, the antibodies, and antibody portions, of the invention, can be used to treat various pulmonary disorders, including adult respiratory distress syndrome (see *e.g.*, PCT Publication No. WO 91/04054), shock lung, chronic pulmonary inflammatory disease, pulmonary sarcoidosis, pulmonary fibrosis and silicosis. The antibody, or antibody portion, may be administered systemically or locally to the lung surface, for example as an aerosol.  
25

#### G. Intestinal Disorders

Tumor necrosis factor has been implicated in the pathophysiology of inflammatory bowel disorders (see *e.g.*, Tracy, K.J., *et al.* (1986) *Science* 234:470-474; Sun, X-M., *et al.*  
30 (1988) *J. Clin. Invest.* 81:1328-1331; MacDonald, T.T., *et al.* (1990) *Clin. Exp. Immunol.* 81:301-305). Chimeric murine anti-hTNF $\alpha$  antibodies have undergone clinical testing for treatment of Crohn's disease (van Dulleman, H.M., *et al.* (1995) *Gastroenterology* 109:129-135). The human antibodies, and antibody portions, of the invention, also can be used to treat intestinal disorders, such as idiopathic inflammatory bowel disease, which includes two  
35 syndromes, Crohn's disease and ulcerative colitis.

#### H. Cardiac Disorders

The antibodies, and antibody portions, of the invention, also can be used to treat various cardiac disorders, including ischemia of the heart (see *e.g.*, European Patent

Application Publication No. EP 453 898) and heart insufficiency (weakness of the heart muscle)(see e.g., PCT Publication No. WO 94/20139).

# I. Others

- 5 The antibodies, and antibody portions, of the invention, also can be used to treat various other disorders in which TNF $\alpha$  activity is detrimental. Examples of other diseases and disorders in which TNF $\alpha$  activity has been implicated in the pathophysiology, and thus which can be treated using an antibody, or antibody portion, of the invention, include inflammatory bone disorders and bone resorption disease (see e.g., Bertolini, D.R., et al. (1986) *Nature* 319:516-518; Konig, A., et al. (1988) *J. Bone Miner. Res.* 3:621-627; Lerner, U.H. and Ohlin, A. (1993) *J. Bone Miner. Res.* 8:147-155; and Shankar, G. and Stern, P.H. (1993) *Bone* 14:871-876), hepatitis, including alcoholic hepatitis (see e.g., McClain, C.J. and Cohen, D.A. (1989) *Hepatology* 9:349-351; Felver, M.E., et al. (1990) *Alcohol. Clin. Exp. Res.* 14:255-259; and Hansen, J., et al. (1994) *Hepatology* 20:461-474) and viral hepatitis (Sheron, N., et al. (1991) *J. Hepatol.* 12:241-245; and Hussain, M.J., et al. (1994) *J. Clin. Pathol.* 47:1112-1115), coagulation disturbances (see e.g., van der Poll, T., et al. (1990) *N. Engl. J. Med.* 322:1622-1627; and van der Poll, T., et al. (1991) *Prog. Clin. Biol. Res.* 367:55-60), burns (see e.g., Giroir, B.P., et al. (1994) *Am. J. Physiol.* 267:H118-124; and Liu, X.S., et al. (1994) *Burns* 20:40-44), reperfusion injury (see e.g., Scales, W.E., et al. (1994) *Am. J. Physiol.* 267:G1122-1127; Serrick, C., et al. (1994) *Transplantation* 58:1158-1162; and Yao, Y.M., et al. (1995) *Resuscitation* 29:157-168), keloid formation (see e.g., McCauley, R.L., et al. (1992) *J. Clin. Immunol.* 12:300-308), scar tissue formation and pyrexia.

- 25 This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application are hereby incorporated by reference.

## **EXAMPLE 1: Kinetic Analysis of Binding of Human Antibodies to hTNF $\alpha$**

- 30 Real-time binding interactions between ligand (biotinylated recombinant human TNF $\alpha$  (hTNF $\alpha$ ) immobilized on a biosensor matrix) and analyte (antibodies in solution) were measured by surface plasmon resonance (SPR) using the BLAcore system (Pharmacia Biosensor, Piscataway, NJ). The system utilizes the optical properties of SPR to detect alterations in protein concentrations within a dextran biosensor matrix. Proteins are covalently bound to the dextran matrix at known concentrations. Antibodies are injected through the dextran matrix and specific binding between injected antibodies and immobilized ligand results in an increased matrix protein concentration and resultant change in the SPR signal. These changes in SPR signal are recorded as resonance units (RU) and are displayed with respect to time along the y-axis of a sensorgram.

To facilitate immobilization of biotinylated rhTNF $\alpha$  on the biosensor matrix, streptavidin is covalently linked via free amine groups to the dextran matrix by first activating carboxyl groups on the matrix with 100 mM N-hydroxysuccinimide (NHS) and 400 mM N-ethyl-N'-(3-diethylaminopropyl) carbodiimide hydrochloride (EDC). Next, streptavidin is injected across the activated matrix. Thirty-five microliters of streptavidin (25  $\mu$ g/ml), diluted in sodium acetate, pH 4.5, is injected across the activated biosensor and free amines on the protein are bound directly to the activated carboxyl groups. Unreacted matrix EDC-esters are deactivated by an injection of 1 M ethanolamine. Streptavidin-coupled biosensor chips also are commercially available (Pharmacia BR-1000-16, Pharmacia Biosensor, Piscataway, NJ).

Biotinylated rhTNF $\alpha$  was prepared by first dissolving 5.0 mg of biotin (D-biotinyl- $\epsilon$ -aminocaproic acid N-hydroxysuccinimide ester; Boehringer Mannheim Cat. No. 1008 960) in 500  $\mu$ l dimethylsulfoxide to make a 10 mg/ml solution. Ten microliters of biotin was added per ml of rhTNF $\alpha$  (at 2.65 mg/ml) for a 2:1 molar ratio of biotin to rhTNF $\alpha$ . The reaction was mixed gently and incubated for two hours at room temperature in the dark. A PD-10 column, Sephadex G-25M (Pharmacia Catalog No. 17-0851-01) was equilibrated with 25 ml of cold PBS and loaded with 2 ml of rhTNF $\alpha$ -biotin per column. The column was eluted with 10  $\times$  1 ml cold PBS. Fractions were collected and read at OD280 (1.0 OD = 1.25 mg/ml). The appropriate fractions were pooled and stored at -80 $^{\circ}$  C until use. Biotinylated rhTNF $\alpha$  also is commercially available (R & D Systems Catalog No. FTA00, Minneapolis, MN).

Biotinylated rhTNF $\alpha$  to be immobilized on the matrix via streptavidin was diluted in PBS running buffer (Gibco Cat. No. 14190-144, Gibco BRL, Grand Island, NY) supplemented with 0.05% (BIAcore) surfactant P20 (Pharmacia BR-1000-54, Pharmacia Biosensor, Piscataway, NJ). To determine the capacity of rhTNF $\alpha$ -specific antibodies to bind immobilized rhTNF $\alpha$ , a binding assay was conducted as follows. Aliquots of biotinylated rhTNF $\alpha$  (25 nM; 10  $\mu$ l aliquots) were injected through the streptavidin-coupled dextran matrix at a flow rate of 5  $\mu$ l/min. Before injection of the protein and immediately afterward, PBS buffer alone flowed through each flow cell. The net difference in signal between baseline and approximately 30 sec. after completion of biotinylated rhTNF $\alpha$  injection was taken to represent the binding value (approximately 500 RU). Direct rhTNF $\alpha$ -specific antibody binding to immobilized biotinylated rhTNF $\alpha$  was measured. Antibodies (20  $\mu$ g/ml) were diluted in PBS running buffer and 25  $\mu$ l aliquots were injected through the immobilized protein matrices at a flow rate of 5  $\mu$ l/min. Prior to injection of antibody, and immediately afterwards, PBS buffer alone flowed through each flow cell. The net difference in baseline signal and signal after completion of antibody injection was taken to represent the binding value of the particular sample. Biosensor matrices were regenerated using 100 mM HCl before injection of the next sample. To determine the off rate ( $K_{off}$ ), on rate ( $K_{on}$ ),

association rate ( $K_a$ ) and dissociation rate ( $K_d$ ) constants, BIAcore kinetic evaluation software (version 2.1) was used.

Representative results of D2E7 (full-length antibody) binding to biotinylated rhTNF $\alpha$ , as compared to the mouse mAb MAK 195 (F(ab')<sub>2</sub> fragment), are shown below in Table 1.

5

Table 1: Binding of D2E7 or MAK 195 to Biotinylated rhTNF $\alpha$

Antibody	[Ab], nM	rhTNF $\alpha$ , bound, RUs	Ab, bound, RUs	rhTNF $\alpha$ /Ab	$K_{off}$ , sec <sup>-1</sup> , (Avg)
D2E7	267	373	1215	1.14	$8.45 \times 10^{-5}$
	133	420	1569	1.30	$5.42 \times 10^{-5}$
	67	434	1633	1.31	$4.75 \times 10^{-5}$
	33	450	1532	1.19	$4.46 \times 10^{-5}$
	17	460	1296	0.98	$3.47 \times 10^{-5}$
	8	486	936	0.67	$2.63 \times 10^{-5}$
	4	489	536	0.38	$2.17 \times 10^{-5}$
	2	470	244	0.18	$3.68 \times 10^{-5}$
					$(4.38 \times 10^{-5})$
MAK 195	400	375	881	1.20	$5.38 \times 10^{-5}$
	200	400	1080	1.38	$4.54 \times 10^{-5}$
	100	419	1141	1.39	$3.54 \times 10^{-5}$
	50	427	1106	1.32	$3.67 \times 10^{-5}$
	25	446	957	1.09	$4.41 \times 10^{-5}$
	13	464	708	0.78	$3.66 \times 10^{-5}$
	6	474	433	0.47	$7.37 \times 10^{-5}$
	3	451	231	0.26	$6.95 \times 10^{-5}$
					$(4.94 \times 10^{-5})$

### EXAMPLE 2: Alanine Scanning Mutagenesis of D2E7 CDR3 Domains

A series of single alanine mutations were introduced by standard methods along the CDR3 domain of the D2E7 VL and the D2E7 VH regions. The light chain mutations are illustrated in Figure 1B (LD2E7\*.A1, LD2E7\*.A3, LD2E7\*.A4, LD2E7\*.A5, LD2E7\*.A7 and LD2E7\*.A8, having an alanine mutation at position 1, 3, 4, 5, 7 or 8, respectively, of the D2E7 VL CDR3 domain). The heavy chain mutations are illustrated in Figure 2B (HD2E7\*.A1, HD2E7\*.A2, HD2E7\*.A3, HD2E7\*.A4, HD2E7\*.A5, HD2E7\*.A6, HD2E7\*.A7, HD2E7\*.A8 and HD2E7\*.A9, having an alanine mutation at position 2, 3, 4, 5, 6, 8, 9, 10 or 11, respectively, of the D2E7 VH CDR3 domain). The kinetics of rhTNF $\alpha$  interaction with an antibody composed of wild-type D2E7 VL and VH was compared to that of antibodies composed of 1) a wild-type D2E7 VL paired with an alanine-substituted D2E7 VH; 2) a wild-type D2E7 VH paired with an alanine-substituted D2E7 VL; or 3) an alanine-

10

15

substituted D2E7 VL paired with an alanine-substituted D2E7 VH. All antibodies were tested as full-length, IgG4 molecules.

Kinetics of interaction of antibodies with rhTNF $\alpha$  was determined by surface plasmon resonance as described in Example 1. The  $K_{off}$  rates for the different VH/VL pairs are summarized below in Table 2:

Table 2: Binding of D2E7 Alanine-Scan Mutants to Biotinylated rhTNF $\alpha$

VH	VL	$K_{off}(\text{sec}^{-1})$
D2E7 VH	D2E7 VL	$9.65 \times 10^{-5}$
HD2E7*.A1	D2E7 VL	$1.4 \times 10^{-4}$
HD2E7*.A2	D2E7 VL	$4.6 \times 10^{-4}$
HD2E7*.A3	D2E7 VL	$8.15 \times 10^{-4}$
HD2E7*.A4	D2E7 VL	$1.8 \times 10^{-4}$
HD2E7*.A5	D2E7 VL	$2.35 \times 10^{-4}$
HD2E7*.A6	D2E7 VL	$2.9 \times 10^{-4}$
HD2E7*.A7	D2E7 VL	$1.0 \times 10^{-4}$
HD2E7*.A8	D2E7 VL	$3.1 \times 10^{-4}$
HD2E7*.A9	D2E7 VL	$8.1 \times 10^{-4}$
D2E7 VH	LD2E7*.A1	$6.6 \times 10^{-5}$
D2E7 VH	LD2E7*.A3	NOT DETECTABLE
D2E7 VH	LD2E7*.A4	$1.75 \times 10^{-4}$
D2E7 VH	LD2E7*.A5	$1.8 \times 10^{-4}$
D2E7 VH	LD2E7*.A7	$1.4 \times 10^{-4}$
D2E7 VH	LD2E7*.A8	$3.65 \times 10^{-4}$
HD2E7*.A9	LD2E7*.A1	$1.05 \times 10^{-4}$

These results demonstrate that the majority of positions of the CDR3 domains of the D2E7 VL region and VH region are amenable to substitution with a single alanine residue. Substitution of a single alanine at position 1, 4, 5, or 7 of the D2E7 VL CDR3 domain or at position 2, 5, 6, 8, 9 or 10 of the D2E7 VH CDR3 domain does not significantly affect the off rate of hTNF $\alpha$  binding as compared to the wild-type parental D2E7 antibody. Substitution of alanine at position 8 of the D2E7 VL CDR3 or at position 3 of the D2E7 VH CDR3 gives a 4-fold faster  $K_{off}$  and an alanine substitution at position 4 or 11 of D2E7 VH CDR3 gives an 8-fold faster  $K_{off}$ , indicating that these positions are more critical for binding to hTNF $\alpha$ . However, a single alanine substitution at position 1, 4, 5, 7 or 8 of the D2E7 VL CDR3 domain or at position 2, 3, 4, 5, 6, 8, 9, 10 or 11 of the D2E7 VH CDR3 domain still results in an anti-hTNF $\alpha$  antibody having a  $K_{off}$  of  $1 \times 10^{-3} \text{ sec}^{-1}$  or less.

### EXAMPLE 3: Binding Analysis of D2E7-Related Antibodies

A series of antibodies related in sequence to D2E7 were analyzed for their binding to rhTNF $\alpha$ , as compared to D2E7, by surface plasmon resonance as described in Example 1. The amino acid sequences of the VL regions tested are shown in Figures 1A and 1B. The amino acid sequences of the VH regions tested are shown in Figures 2A and 2B. The  $K_{off}$  rates for various VH/VL pairs (in the indicated format, either as a full-length IgG1 or IgG4 antibody or as a scFv) are summarized below in Table 3:

Table 3: Binding of D2E7-Related Antibodies to Biotinylated rhTNF $\alpha$

VH	VL	Format	$K_{off}$ (sec <sup>-1</sup> )
D2E7 VH	D2E7 VL	IgG1/IgG4	$9.65 \times 10^{-5}$
VH1-D2	LOE7	IgG1/IgG4	$7.7 \times 10^{-5}$
VH1-D2	LOE7	scFv	$4.6 \times 10^{-4}$
VH1-D2.N	LOE7.T	IgG4	$2.1 \times 10^{-5}$
VH1-D2.Y	LOE7.A	IgG4	$2.7 \times 10^{-5}$
VH1-D2.N	LOE7.A	IgG4	$3.2 \times 10^{-5}$
VH1-D2	EP B12	scFv	$8.0 \times 10^{-4}$
VH1-D2	2SD4 VL	scFv	$1.94 \times 10^{-3}$
3C-H2	LOE7	scFv	$1.5 \times 10^{-3}$
2SD4 VH	LOE7	scFv	$6.07 \times 10^{-3}$
2SD4 VH	2SD4 VL	scFv	$1.37 \times 10^{-2}$
VH1A11	2SD4 VL	scFv	$1.34 \times 10^{-2}$
VH1B12	2SD4 VL	scFv	$1.01 \times 10^{-2}$
VH1B11	2SD4 VL	scFv	$9.8 \times 10^{-3}$
VH1E4	2SD4 VL	scFv	$1.59 \times 10^{-2}$
VH1F6	2SD4 VL	scFv	$2.29 \times 10^{-2}$
VH1D8	2SD4 VL	scFv	$9.5 \times 10^{-3}$
VH1G1	2SD4 VL	scFv	$2.14 \times 10^{-2}$
2SD4 VH	EP B12	scFv	$6.7 \times 10^{-3}$
2SD4 VH	VL10E4	scFv	$9.6 \times 10^{-3}$
2SD4 VH	VL100A9	scFv	$1.33 \times 10^{-2}$
2SD4 VH	VL100D2	scFv	$1.41 \times 10^{-2}$
2SD4 VH	VL10F4	scFv	$1.11 \times 10^{-2}$
2SD4 VH	VLLOE5	scFv	$1.16 \times 10^{-2}$
2SD4 VH	VLL0F9	scFv	$6.09 \times 10^{-3}$
2SD4 VH	VLL0F10	scFv	$1.34 \times 10^{-2}$
2SD4 VH	VLL0G7	scFv	$1.56 \times 10^{-2}$
2SD4 VH	VLL0G9	scFv	$1.46 \times 10^{-2}$
2SD4 VH	VLL0H1	scFv	$1.17 \times 10^{-2}$
2SD4 VH	VLL0H10	scFv	$1.12 \times 10^{-2}$
2SD4 VH	VL1B7	scFv	$1.3 \times 10^{-2}$
2SD4 VH	VL1C1	scFv	$1.36 \times 10^{-2}$
2SD4 VH	VL1C7	scFv	$2.0 \times 10^{-2}$
2SD4 VH	VL0.1F4	scFv	$1.76 \times 10^{-2}$
2SD4 VH	VL0.1H8	scFv	$1.14 \times 10^{-2}$

The slow off rates (*i.e.*,  $K_{\text{off}} \leq 1 \times 10^{-4} \text{ sec}^{-1}$ ) for full-length antibodies (*i.e.*, IgG format) having a VL selected from D2E7, LOE7, LOE7.T and LOE7.A, which have either a threonine or an alanine at position 9, indicate that position 9 of the D2E7 VL CDR3 can be occupied by either of these two residues without substantially affecting the  $K_{\text{off}}$ . Accordingly, a consensus motif for the D2E7 VL CDR3 comprises the amino acid sequence: Q-R-Y-N-R-A-P-Y-(T/A) (SEQ ID NO: 3). Furthermore, the slow off rates (*i.e.*,  $K_{\text{off}} \leq 1 \times 10^{-4} \text{ sec}^{-1}$ ) for antibodies having a VH selected from D2E7, VH1-D2.N and VH1-D2.Y, which have either a tyrosine or an asparagine at position 12, indicate that position 12 of the D2E7 VH CDR3 can be occupied by either of these two residues without substantially affecting the  $K_{\text{off}}$ . Accordingly, a consensus motif for the D2E7 VH CDR3 comprises the amino acid sequence: V-S-Y-L-S-T-A-S-S-L-D-(Y/N) (SEQ ID NO: 4).

The results shown in Table 3 demonstrate that, in scFv format, antibodies containing the 2SD4 VL or VH CDR3 region exhibit a faster  $K_{\text{off}}$  (*i.e.*,  $K_{\text{off}} \geq 1 \times 10^{-3} \text{ sec}^{-1}$ ) as compared to antibodies containing the D2E7 VL or VH CDR3 region. Within the VL CDR3, 2SD4 differs from D2E7 at positions 2, 5 and 9. As discussed above, however, position 9 may be occupied by Ala (as in 2SD4) or Thr (as in D2E7) without substantially affecting the  $K_{\text{off}}$ . Thus, by comparison of 2SD4 and D2E7, positions 2 and 5 of the D2E7 VL CDR3, both arginines, can be identified as being critical for the association of the antibody with hTNF $\alpha$ . These residues could be directly involved as contact residues in the antibody binding site or could contribute critically to maintaining the scaffolding architecture of the antibody molecule in this region. Regarding the importance of position 2, replacement of Arg (in LOE7, which has the same VL CDR3 as D2E7) with Lys (in EP B12) accelerates the off rate by a factor of two. Regarding the importance of position 5, replacement of Arg (in D2E7) with Ala (in LD2E7\*.A5), as described in Example 2, also accelerates the off rate two-fold. Furthermore, without either Arg at positions 2 and 5 (in 2SD4), the off rate is five-fold faster. However, it should be noted that although position 5 is important for improved binding to hTNF $\alpha$ , a change at this position can be negated by changes at other positions, as seen in VLLOE4, VLLOH1 or VL0.1H8.

Within the VH CDR3, 2SD4 differs from D2E7 at positions 1, 7 and 12. As discussed above, however, position 12 may be occupied by Asn (as in 2SD4) or Tyr (as in D2E7) without substantially affecting the  $K_{\text{off}}$ . Thus, by comparison of 2SD4 and D2E7, positions 1 and 7 of the D2E7 VH CDR3 can be identified as being critical for binding to hTNF $\alpha$ . As discussed above, these residues could be directly involved as contact residues in the antibody binding site or could contribute critically to maintaining the scaffolding architecture of the antibody molecule in this region. Both positions are important for binding to hTNF $\alpha$  since when the 3C-H2 VH CDR3 (which has a valine to alanine change at position 1 with respect to the D2E7 VH CDR3) is used, the scFv has a 3-fold faster off rate than when the D2E7 VH CDR3 is used but this off rate is still four times slower than when the 2SD4

VH CDR3 is used (which has changes at both positions 1 and 7 with respect to the D2E7 VH CDR3).

#### EXAMPLE 4: Functional Activity of D2E7

- 5 To examine the functional activity of D2E7, the antibody was used in several assays that measure the ability of the antibody to inhibit hTNF $\alpha$  activity, either *in vitro* or *in vivo*.

##### A. Neutralization of TNF $\alpha$ -Induced Cytotoxicity in L929 Cells

- 10 Human recombinant TNF $\alpha$  (rhTNF $\alpha$ ) causes cell cytotoxicity to murine L929 cells after an incubation period of 18-24 hours. Human anti-hTNF $\alpha$  antibodies were evaluated in L929 assays by coinubation of antibodies with rhTNF $\alpha$  and the cells as follows. A 96-well microtiter plate containing 100  $\mu$ l of anti-hTNF $\alpha$  Abs was serially diluted 1/3 down the plate in duplicates using RPMI medium containing 10% fetal bovine serum (FBS). Fifty
- 15 microliters of rhTNF $\alpha$  was added for a final concentration of 500 pg/ml in each sample well. The plates were then incubated for 30 minutes at room temperature. Next, 50  $\mu$ l of TNF $\alpha$ -sensitive L929 mouse fibroblasts cells were added for a final concentration of  $5 \times 10^4$  cells per well, including 1  $\mu$ g/ml Actinomycin-D. Controls included medium plus cells and rhTNF $\alpha$  plus cells. These controls, and a TNF $\alpha$  standard curve, ranging from 2 ng/ml to 8.2
- 20 pg/ml, were used to determine the quality of the assay and provide a window of neutralization. The plates were then incubated overnight (18-24 hours) at 37° C in 5% CO $_2$ .

- One hundred microliters of medium was removed from each well and 50  $\mu$ l of 5 mg/ml 3,(4,4-dimethylthiazol-2-yl)2,5-diphenyl-tetrazolium bromide (MTT; commercially available from Sigma Chemical Co., St. Louis, MO) in PBS was added. The plates were then
- 25 incubated for 4 hours at 37° C. Fifty microliters of 20% sodium dodecyl sulfate (SDS) was then added to each well and the plates were incubated overnight at 37° C. The optical density at 570/630 nm was measured, curves were plotted for each sample and IC $_{50}$ s were determined by standard methods.

- Representative results for human antibodies having various VL and VH pairs, as
- 30 compared to the murine MAK 195 mAb, are shown in Figure 3 and in Table 4 below.

Table 4: Neutralization of TNF $\alpha$ -Induced L929 Cytotoxicity

VH	VL	Structure	IC $_{50}$ M
D2E7	D2E7	scFv	$1.1 \times 10^{-10}$
D2E7	D2E7	IgG4	$4.7 \times 10^{-11}$
2SD4	2SD4	scFv/IgG1/IgG4	$3.0 \times 10^{-7}$
2SD4	LOE7	scFv	$4.3 \times 10^{-8}$
VH1-D2	2SD4	scFv	$1.0 \times 10^{-8}$
VH1-D2	LOE7	scFv/IgG1/IgG4	$3.4 \times 10^{-10}$



VH1.D2.Y	LOE7.T	IgG4	$8.1 \times 10^{-11}$
VH1-D2.N	LOE7.T	IgG4	$1.3 \times 10^{-10}$
VH1-D2.Y	LOE7.A	IgG4	$2.8 \times 10^{-11}$
VH1-D2.N	LOE7.A	IgG4	$6.2 \times 10^{-11}$
MAK 195	MAK 195	scFv	$1.9 \times 10^{-8}$
MAK 195	MAK 195	F(ab') <sub>2</sub>	$6.2 \times 10^{-11}$

The results in Figure 3 and Table 4 demonstrate that the D2E7 human anti-hTNF $\alpha$  antibody, and various D2E7-related antibodies, neutralize TNF $\alpha$ -induced L929 cytotoxicity with a capacity approximately equivalent to that of the murine anti-hTNF $\alpha$  mAb MAK 195.

5

#### B. Inhibition of TNF $\alpha$ Binding to TNF $\alpha$ Receptors on U-937 Cells

The ability of human anti-hTNF $\alpha$  antibodies to inhibit the binding of hTNF $\alpha$  to hTNF $\alpha$  receptors on the surface of cells was examined using the U-937 cell line (ATCC No. CRL 1593), a human histiocytic cell line that expresses hTNF $\alpha$  receptors. U-937 cells were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum (Hyclone A-1111, Hyclone Laboratories, Logan, UT), L-glutamine (4 mM), HEPES buffer solution (10 mM), penicillin (100  $\mu$ g/ml) and streptomycin (100  $\mu$ g/ml). To examine the activity of full-length IgG antibodies, U-937 cells were preincubated with PBS supplemented with 1 mg/ml of human IgG (Sigma I-4506, Sigma Chemical Co., St. Louis, MO) for 45 minutes on ice and then cells were washed three times with binding buffer. For the receptor binding assay, U-937 cells ( $5 \times 10^6$  cells/well) were incubated in a binding buffer (PBS supplemented with 0.2% bovine serum albumin) in 96-well microtiter plates (Costar 3799, Costar Corp., Cambridge, MA) together with  $^{125}$ I-labeled rhTNF $\alpha$  ( $3 \times 10^{-10}$  M; 25  $\mu$ Ci/ml; obtained from NEN Research Products, Wilmington, DE), with or without anti-hTNF $\alpha$  antibodies, in a total volume of 0.2 ml. The plates were incubated on ice for 1.5 hours. Then, 75  $\mu$ l of each sample was transferred to 1.0 ml test tubes (Sarstedt 72.700, Sarstedt Corp., Princeton, NJ) containing dibutylphthalate (Sigma D-2270, Sigma Chemical Co., St. Louis, MO) and dinonylphthalate (ICN 210733, ICN, Irvine, CA). The test tubes contained a 300  $\mu$ l mixture of dibutylphthalate and dinonylphthalate, 2:1 volume ratio, respectively. Free (*i.e.*, unbound)  $^{125}$ I-labeled rhTNF $\alpha$  was removed by microcentrifugation for five minutes. Then, each test tube end containing a cell pellet was cut with the aid of a microtube scissor (Bel-Art 210180001, Bel-Art Products, Pequannock, NJ). The cell pellet contains  $^{125}$ I-labeled rhTNF $\alpha$  bound to the p60 or p80 TNF $\alpha$  receptor, whereas the aqueous phase above the oil mixture contains excess free  $^{125}$ I-labeled rhTNF $\alpha$ . All cell pellets were collected in a counting tube (Falcon 2052, Becton Dickinson Labware, Lincoln Park, NJ) and counted in a scintillation counter.

30

Representative results are shown in Figure 4. The  $IC_{50}$  value for D2E7 inhibition of hTNF $\alpha$  binding to hTNF $\alpha$  receptors on U-937 cells is approximately  $3 \times 10^{-10}$  M. These results demonstrate that the D2E7 human anti-hTNF $\alpha$  antibody inhibits rhTNF $\alpha$  binding to hTNF $\alpha$  receptors on U-937 cells at concentrations approximately equivalent to that of the murine anti-hTNF $\alpha$  mAb MAK 195.

### C. Inhibition of ELAM-1 Expression on HUVEC

Human umbilical vein endothelial cells (HUVEC) can be induced to express endothelial cell leukocyte adhesion molecule 1 (ELAM-1) on their cell-surface by treatment with rhTNF $\alpha$ , which can be detected by reacting rhTNF $\alpha$ -treated HUVEC with an mouse anti-human ELAM-1 antibody. The ability of human anti-hTNF $\alpha$  antibodies to inhibit this TNF $\alpha$ -induced expression of ELAM-1 on HUVEC was examined as follows: HUVEC (ATCC No. CRL 1730) were plated in 96-well plates ( $5 \times 10^4$  cells/well) and incubated overnight at 37 °C. The following day, serial dilutions of human anti-hTNF $\alpha$  antibody (1:10) were prepared in a microtiter plate, starting with 20-100  $\mu$ g/ml of antibody. A stock solution of rhTNF $\alpha$  was prepared at 4.5 ng/ml, aliquots of rhTNF $\alpha$  were added to each antibody-containing well and the contents were mixed well. Controls included medium alone, medium plus anti-hTNF $\alpha$  antibody and medium plus rhTNF $\alpha$ . The HUVEC plates were removed from their overnight incubation at 37° C and the medium gently aspirated from each well. Two hundred microliters of the antibody-rhTNF $\alpha$  mixture were transferred to each well of the HUVEC plates. The HUVEC plates were then further incubated at 37° C for 4 hours. Next, a murine anti-ELAM-1 antibody stock was diluted 1:1000 in RPMI. The medium in each well of the HUVEC plate was gently aspirated, 50  $\mu$ l/well of the anti-ELAM-1 antibody solution was added and the HUVEC plates were incubated 60 minutes at room temperature. An  $^{125}$ I-labeled anti-mouse Ig antibody solution was prepared in RPMI (approximately 50,000 cpm in 50  $\mu$ l). The medium in each well of the HUVEC plates was gently aspirated, the wells were washed twice with RPMI and 50  $\mu$ l of the  $^{125}$ I-labeled anti-mouse Ig solution was added to each well. The plates were incubated for one hour at room temperature and then each well was washed three times with RPMI. One hundred eighty microliters of 5% SDS was added to each well to lyse the cells. The cell lysate from each well was then transferred to a tube and counted in a scintillation counter.

Representative results are shown in Figure 5. The  $IC_{50}$  value for D2E7 inhibition of hTNF $\alpha$ -induced expression of ELAM-1 on HUVEC is approximately  $6 \times 10^{-11}$  M. These results demonstrate that the D2E7 human anti-hTNF $\alpha$  antibody inhibits the hTNF $\alpha$ -induced expression of ELAM-1 on HUVEC at concentrations approximately equivalent to that of the murine anti-hTNF $\alpha$  mAb MAK 195.

#### D. In Vivo Neutralization of hTNF $\alpha$

Injection of recombinant human TNF $\alpha$  (rhTNF $\alpha$ ) to D-galactosamine sensitized mice causes lethality within a 24 hour time period. TNF $\alpha$  neutralizing agents have been shown to prevent lethality in this model. To examine the ability of human anti-hTNF $\alpha$  antibodies to neutralize hTNF $\alpha$  *in vivo* in this model, C57Bl/6 mice were injected with varying concentrations of D2E7-IgG1, or a control protein, in PBS intraperitoneally (i.p.). Mice were challenged 30 minutes later with 1  $\mu$ g of rhTNF $\alpha$  and 20 mg of D-galactosamine in PBS i.p., and observed 24 hours later. These amount of rhTNF $\alpha$  and D-galactosamine were previously determined to achieve 80-90% lethality in these mice.

Representative results, depicted as a bar graph of % survival versus antibody concentration, are shown in Figure 6. The black bars represent D2E7, whereas the hatched bars represent MAK 195. Injection of 2.5-25  $\mu$ g of D2E7 antibody per mouse protected the animals from TNF $\alpha$ -induced lethality. The ED<sub>50</sub> value is approximately 1-2.5  $\mu$ g/mouse. The positive control antibody, MAK 195, was similar in its protective ability. Injection of D2E7 in the absence of rhTNF $\alpha$  did not have any detrimental effect on the mice. Injection of a non-specific human IgG1 antibody did not offer any protection from TNF $\alpha$ -induced lethality.

Additionally, in a hTNF $\alpha$ -mediated rabbit pyrexia model, D2E7 antibody attenuated the temperature rise due to administration of hTNF $\alpha$  in a dose dependent manner.

These results demonstrate that D2E7 is effective at inhibiting hTNF $\alpha$  activity *in vivo*.

#### E. D2E7 Neutralization of TNF $\alpha$ s from Other Species

The binding specificity of D2E7 was examined by measuring its ability to neutralize tumor necrosis factors from various primate species and from mouse, using an L929 cytotoxicity assay (as described in Example 4, subsection A, above). The results are summarized in Table 5 below:

Table 5: D2E7 Neutralization of TNF $\alpha$  from Different Species

TNF $\alpha$ *	IC <sub>50</sub> for D2E7 Neutralization (M)**
Human	6.0 x 10 <sup>-11</sup>
Chimpanzee	5.5 x 10 <sup>-11</sup>
baboon	6.0 x 10 <sup>-11</sup>
marmoset	4.0 x 10 <sup>-11</sup>
cynomolgus	8.0 x 10 <sup>-11</sup>
rhesus	~3 x 10 <sup>-11</sup>
mouse	6.0 x 10 <sup>-8</sup>

The results in Table 5 demonstrate that D2E7 can neutralize the activity of five primate TNF $\alpha$ s approximately equivalently to human TNF $\alpha$  and, moreover, can neutralize the activity of mouse TNF $\alpha$ , albeit ~1000-fold less well than human TNF $\alpha$ . However, the IC<sub>50</sub> for neutralization of mouse TNF $\alpha$  ( $6.0 \times 10^{-8}$  M) is still significant, as it compares to that of a hamster anti-mouse TNF $\alpha$  antibody commercially available (Genzyme Corp.) for detecting or inhibiting mouse TNF $\alpha$ . Moreover, D2E7 was found to bind to porcine TNF $\alpha$  but did not specifically bind to other cytokines, such as lymphotoxin (TNF $\beta$ ), IL-1 $\alpha$ , IL-1 $\beta$ , IL-2, IL-4, IL-6, IL-8, IFN $\gamma$  and TGF $\beta$ .

10

Forming part of the present disclosure is the appended Sequence Listing, the contents of which are summarized in the table below:

SEQ ID NO:	ANTIBODY CHAIN	REGION	SEQUENCE TYPE
1	D2E7	VL	amino acid
2	D2E7	VH	amino acid
3	D2E7	VL CDR3	amino acid
4	D2E7	VH CDR3	amino acid
5	D2E7	VL CDR2	amino acid
6	D2E7	VH CDR2	amino acid
7	D2E7	VL CDR1	amino acid
8	D2E7	VH CDR1	amino acid
9	2SD4	VL	amino acid
10	2SD4	VH	amino acid
11	2SD4	VL CDR3	amino acid
12	EP B12	VL CDR3	amino acid
13	VL10E4	VL CDR3	amino acid
14	VL100A9	VL CDR3	amino acid
15	VLL100D2	VL CDR3	amino acid
16	VLL0F4	VL CDR3	amino acid
17	LOE5	VL CDR3	amino acid
18	VLOG7	VL CDR3	amino acid
19	VLOG9	VL CDR3	amino acid
20	VLOH1	VL CDR3	amino acid
21	VLOH10	VL CDR3	amino acid
22	VL1B7	VL CDR3	amino acid
23	VL1C1	VL CDR3	amino acid
24	VL0.1F4	VL CDR3	amino acid
25	VL0.1H8	VL CDR3	amino acid
26	LOE7.A	VL CDR3	amino acid
27	2SD4	VH CDR3	amino acid
28	VH1B11	VH CDR3	amino acid

29	VH1D8	VH CDR3	amino acid
30	VH1A11	VH CDR3	amino acid
31	VH1B12	VH CDR3	amino acid
32	VH1E4	VH CDR3	amino acid
33	VH1F6	VH CDR3	amino acid
34	3C-H2	VH CDR3	amino acid
35	VH1-D2.N	VH CDR3	amino acid
36	D2E7	VL	nucleic acid
37	D2E7	VH	nucleic acid

### EQUIVALENTS

- Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- 5 (i) APPLICANTS: Salfeld, Jochen G.  
Allen, Deborah J.  
Hoogenboom, Hendricus R.J.M.  
Kaymakcalan, Zehra  
Labkovsky, Boris  
10 Mankovich, John A.  
McGuinness, Brian T.  
Roberts, Andrew J.  
Sakorafas, Paul  
Schoenhaut, David  
15 Vaughan, Tristan J.  
White, Michael  
Wilton, Andrew J.
- (ii) TITLE OF INVENTION: Human Antibodies that Bind Human TNF $\alpha$
- 20 (iii) NUMBER OF SEQUENCES: 37
- (iv) CORRESPONDENCE ADDRESS:  
25 (A) ADDRESSEE: LAHIVE & COCKFIELD  
(B) STREET: 60 State Street, suite 510  
(C) CITY: Boston  
(D) STATE: Massachusetts  
(E) COUNTRY: USA  
(F) ZIP: 02109-1875
- 30 (v) COMPUTER READABLE FORM:  
(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
35 (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:  
(A) APPLICATION NUMBER: US  
40 (B) FILING DATE: 08-FEB-1996  
(C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:  
(A) NAME: DeConti, Giulio A., Jr.  
45 (B) REGISTRATION NUMBER: 31,503  
(C) REFERENCE/DOCKET NUMBER: BBI-043
- (ix) TELECOMMUNICATION INFORMATION:  
(A) TELEPHONE: (617) 227-7400  
50 (B) TELEFAX: (617) 227-5941

(2) INFORMATION FOR SEQ ID NO:1:

- 55 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 107 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly  
1 5 10 15

10 Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Tyr  
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile  
35 40 45

15 Tyr Ala Ala Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly  
50 55 60

20 Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro  
65 70 75 80

Glu Asp Val Ala Thr Tyr Tyr Cys Gln Arg Tyr Asn Arg Ala Pro Tyr  
85 90 95

25 Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys  
100 105

(2) INFORMATION FOR SEQ ID NO:2:

30

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 121 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Arg  
1 5 10 15

45 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr  
20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
35 40 45

50 Ser Ala Ile Thr Trp Asn Ser Gly His Ile Asp Tyr Ala Asp Ser Val  
50 55 60

55 Glu Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr  
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

Ala Lys Val Ser Tyr Leu Ser Thr Ala Ser Ser Leu Asp Tyr Trp Gly  
100 105 110

5 Gln Gly Thr Leu Val Thr Val Ser Ser  
115 120

(2) INFORMATION FOR SEQ ID NO:3:

- 10 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 9 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear
- 15 (ii) MOLECULE TYPE: peptide  
(v) FRAGMENT TYPE: internal
- 20 (ix) FEATURE:  
(A) NAME/KEY: Modified-site  
(B) LOCATION: 9  
(D) OTHER INFORMATION: /note= "Xaa is Thr or Ala"
- 25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:  
Gln Arg Tyr Asn Arg Ala Pro Tyr Xaa  
1 5

30 (2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 12 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear
- 35 (ii) MOLECULE TYPE: peptide  
(v) FRAGMENT TYPE: internal
- 40 (ix) FEATURE:  
(A) NAME/KEY: Modified-site  
(B) LOCATION: 12  
(D) OTHER INFORMATION: /note= "Xaa is Tyr or Asn"
- 45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:  
Val Ser Tyr Leu Ser Thr Ala Ser Ser Leu Asp Xaa  
1 5 10

(2) INFORMATION FOR SEQ ID NO:5:

- 55 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 7 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide



(v) FRAGMENT TYPE: internal

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Ala Ala Ser Thr Leu Gln Ser  
1 5

10 (2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 17 amino acids  
(B) TYPE: amino acid  
15 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ala Ile Thr Trp Asn Ser Gly His Ile Asp Tyr Ala Asp Ser Val Glu  
1 5 10 15  
25 Gly

30 (2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 11 amino acids  
(B) TYPE: amino acid  
35 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Arg Ala Ser Gln Gly Ile Arg Asn Tyr Leu Ala  
1 5 10  
45

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:  
50 (A) LENGTH: 5 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

55 (v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Asp Tyr Ala Met His  
1 5

5 (2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 107 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Ile Gly  
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Tyr  
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile  
35 40 45

Tyr Ala Ala Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly  
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro  
65 70 75 80

Glu Asp Val Ala Thr Tyr Tyr Cys Gln Lys Tyr Asn Ser Ala Pro Tyr  
85 90 95

Ala Phe Gly Gln Gly Thr Lys Val Glu Ile Lys  
100 105

40 (2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 121 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Arg  
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr  
20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Asp Trp Val  
35 40 45

5 Ser Ala Ile Thr Trp Asn Ser Gly His Ile Asp Tyr Ala Asp Ser Val  
50 55 60

Glu Gly Arg Phe Ala Val Ser Arg Asp Asn Ala Lys Asn Ala Leu Tyr  
65 70 75 80

10 Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

Thr Lys Ala Ser Tyr Leu Ser Thr Ser Ser Ser Leu Asp Asn Trp Gly  
100 105 110

15 Gln Gly Thr Leu Val Thr Val Ser Ser  
115 120

20 (2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 9 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Gln Lys Tyr Asn Ser Ala Pro Tyr Ala  
1 5

35 (2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 9 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

50 Gln Lys Tyr Asn Arg Ala Pro Tyr Ala  
1 5

(2) INFORMATION FOR SEQ ID NO:13:

55 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 9 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

5 (v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Gln Lys Tyr Gln Arg Ala Pro Tyr Thr  
1 5

10

(2) INFORMATION FOR SEQ ID NO:14:

15 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 9 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

25 Gln Lys Tyr Ser Ser Ala Pro Tyr Thr  
1 5

(2) INFORMATION FOR SEQ ID NO:15:

30 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 9 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Gln Lys Tyr Asn Ser Ala Pro Tyr Thr  
1 5

45

(2) INFORMATION FOR SEQ ID NO:16:

50 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 9 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

55 (v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Gln Lys Tyr Asn Arg Ala Pro Tyr Thr  
1 5

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Gln Lys Tyr Asn Ser Ala Pro Tyr Tyr  
1 5

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Gln Lys Tyr Asn Ser Ala Pro Tyr Asn  
1 5

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Gln Lys Tyr Thr Ser Ala Pro Tyr Thr  
1 5

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 9 amino acids  
    (B) TYPE: amino acid  
    (D) TOPOLOGY: linear

5

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Gln Lys Tyr Asn Arg Ala Pro Tyr Asn  
1                    5

15 (2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 9 amino acids  
    (B) TYPE: amino acid  
    (D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: peptide

25 (v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Gln Lys Tyr Asn Ser Ala Ala Tyr Ser  
30 1                    5

(2) INFORMATION FOR SEQ ID NO:22:

35 (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 9 amino acids  
    (B) TYPE: amino acid  
    (D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

45 Gln Gln Tyr Asn Ser Ala Pro Asp Thr  
1                    5

50 (2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 9 amino acids  
    (B) TYPE: amino acid  
    (D) TOPOLOGY: linear

55

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

5       Gln Lys Tyr Asn Ser Asp Pro Tyr Thr  
      1                   5

(2) INFORMATION FOR SEQ ID NO:24:

10       (i) SEQUENCE CHARACTERISTICS:  
          (A) LENGTH: 9 amino acids  
          (B) TYPE: amino acid  
          (D) TOPOLOGY: linear

15       (ii) MOLECULE TYPE: peptide

          (v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

20       Gln Lys Tyr Ile Ser Ala Pro Tyr Thr  
      1                   5

25       (2) INFORMATION FOR SEQ ID NO:25:

          (i) SEQUENCE CHARACTERISTICS:  
              (A) LENGTH: 9 amino acids  
              (B) TYPE: amino acid  
30            (D) TOPOLOGY: linear

          (ii) MOLECULE TYPE: peptide

          (v) FRAGMENT TYPE: internal

35       (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

      Gln Lys Tyr Asn Arg Pro Pro Tyr Thr  
      1                   5

40

(2) INFORMATION FOR SEQ ID NO:26:

45       (i) SEQUENCE CHARACTERISTICS:  
          (A) LENGTH: 9 amino acids  
          (B) TYPE: amino acid  
          (D) TOPOLOGY: linear

50       (ii) MOLECULE TYPE: peptide

          (v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

55       Gln Arg Tyr Asn Arg Ala Pro Tyr Ala  
      1                   5

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 12 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Ala Ser Tyr Leu Ser Thr Ser Ser Ser Leu Asp Asn  
1 5 10

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 12 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Ala Ser Tyr Leu Ser Thr Ser Ser Ser Leu Asp Lys  
1 5 10

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 12 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Ala Ser Tyr Leu Ser Thr Ser Ser Ser Leu Asp Tyr  
1 5 10

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 12 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide



(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

5      Ala Ser Tyr Leu Ser Thr Ser Ser Ser Leu Asp Asp  
      1                      5                      10

(2) INFORMATION FOR SEQ ID NO:31:

10      (i) SEQUENCE CHARACTERISTICS:  
          (A) LENGTH: 12 amino acids  
          (B) TYPE: amino acid  
          (D) TOPOLOGY: linear

15      (ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

20      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

      Ala Ser Tyr Leu Ser Thr Ser Phe Ser Leu Asp Tyr  
      1                      5                      10

(2) INFORMATION FOR SEQ ID NO:32:

25      (i) SEQUENCE CHARACTERISTICS:  
          (A) LENGTH: 12 amino acids  
          (B) TYPE: amino acid  
          (D) TOPOLOGY: linear

30      (ii) MOLECULE TYPE: peptide

35      (v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

40      Ala Ser Tyr Leu Ser Thr Ser Ser Ser Leu His Tyr  
      1                      5                      10

(2) INFORMATION FOR SEQ ID NO:33:

45      (i) SEQUENCE CHARACTERISTICS:  
          (A) LENGTH: 12 amino acids  
          (B) TYPE: amino acid  
          (D) TOPOLOGY: linear

50      (ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

55      Ala Ser Phe Leu Ser Thr Ser Ser Ser Leu Glu Tyr  
      1                      5                      10

(2) INFORMATION FOR SEQ ID NO:34:

- 5 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 12 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- 10 (v) FRAGMENT TYPE: internal
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:
- 15 Ala Ser Tyr Leu Ser Thr Ala Ser Ser Leu Glu Tyr  
 1 5 10

(2) INFORMATION FOR SEQ ID NO:35:

- 20 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 12 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear
- 25 (ii) MOLECULE TYPE: peptide
- (v) FRAGMENT TYPE: internal
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:
- 30 Val Ser Tyr Leu Ser Thr Ala Ser Ser Leu Asp Asn  
 1 5 10

(2) INFORMATION FOR SEQ ID NO:36:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 321 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- 45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:
- |  |     |
|--|-----|
| GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCCTGCAT CTGTAGGGGA CAGAGTCACC | 60  |
| ATCACTTGTC GGGCAAGTCA GGGCATCAGA AATTACTTAG CCTGGTATCA GCAAAAACCA  | 120 |
| GGGAAAGCCC CTAAGCTCCT GATCTATGCT GCATCCACTT TGCAATCAGG GGTCCCATCT  | 180 |
| CGGTTCACTG GCAGTGGATC TGGGACAGAT TTCACTCTCA CCATCAGCAG CCTACAGCCT  | 240 |
| GAAGATGTIG CAACTTATTA CTGTCAAAGG TATAACCGTG CACCGTATAC TTTTGCCAG   | 300 |
| GGGACCAAGG TGGAAATCAA A  | 321 |

(2) INFORMATION FOR SEQ ID NO 37:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 363 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GAGGTGCAGC TGGTGGAGTC TGGGGGAGGC TTGGTACAGC CCGGCAGGTC CCTGAGACTC 60  
15 TCCTGTGCGG CCTCTGGATT CACCTTTGAT GATTATGCCA TGCACTGGGT CCGGCAAGCT 120  
CCAGGGAAGG GCCTGGAATG GGTCTCAGCT ATCACTTGGA ATAGTGGTCA CATAGACTAT 180  
20 GCGGACTCTG TGGAGGGCCG ATTCACCATC TCCAGAGACA ACGCCAAGAA CTCCTGTAT 240  
CTGCAAATGA ACAGTCTGAG AGCTGAGGAT ACGGCCGTAT ATTACTGTGC GAAAGTCTCG 300  
TACCTTAGCA CCGCGTCCTC CTTGACTAT TGGGGCCAAG GTACCCTGGT CACCGTCTCG 360  
25 AGT 363

## CLAIMS

1. An isolated human antibody, or an antigen-binding portion thereof, that dissociates from human TNF $\alpha$  with a  $K_d$  of  $1 \times 10^{-8}$  M or less and a  $K_{off}$  rate constant of  $1 \times 10^{-3}$  s $^{-1}$  or less, both determined by surface plasmon resonance, and neutralizes human TNF $\alpha$  cytotoxicity in a standard *in vitro* L929 assay with an IC $_{50}$  of  $1 \times 10^{-7}$  M or less.
2. The isolated human antibody, or antigen-binding portion thereof, of claim 1, which dissociates from human TNF $\alpha$  with a  $K_{off}$  rate constant of  $5 \times 10^{-4}$  s $^{-1}$  or less, .
3. The isolated human antibody, or antigen-binding portion thereof, of claim 1, which dissociates from human TNF $\alpha$  with a  $K_{off}$  rate constant of  $1 \times 10^{-4}$  s $^{-1}$  or less.
4. The isolated human antibody, or antigen-binding portion thereof, of claim 1, which neutralizes human TNF $\alpha$  cytotoxicity in a standard *in vitro* L929 assay with an IC $_{50}$  of  $1 \times 10^{-8}$  M or less.
5. The isolated human antibody, or antigen-binding portion thereof, of claim 1, which neutralizes human TNF $\alpha$  cytotoxicity in a standard *in vitro* L929 assay with an IC $_{50}$  of  $1 \times 10^{-9}$  M or less.
6. The isolated human antibody, or antigen-binding portion thereof, of claim 1, which neutralizes human TNF $\alpha$  cytotoxicity in a standard *in vitro* L929 assay with an IC $_{50}$  of  $1 \times 10^{-10}$  M or less.
7. The isolated human antibody, or antigen-binding portion thereof, of claim 1, which is a recombinant antibody, or antigen-binding portion thereof.
8. The isolated human antibody, or antigen-binding portion thereof, of claim 1, which inhibits human TNF $\alpha$ -induced expression of ELAM-1 on human umbilical vein endothelial cells.
9. An isolated human antibody, or antigen-binding portion thereof, with the following characteristics:
  - a) dissociates from human TNF $\alpha$  with a  $K_{off}$  rate constant of  $1 \times 10^{-3}$  s $^{-1}$  or less, as determined by surface plasmon resonance;
  - b) has a light chain CDR3 domain comprising the amino acid sequence of SEQ ID NO: 3, or modified from SEQ ID NO: 3 by a single alanine substitution at position 1, 4, 5, 7

or 8 or by one to five conservative amino acid substitutions at positions 1, 3, 4, 6, 7, 8 and/or 9;

c) has a heavy chain CDR3 domain comprising the amino acid sequence of SEQ ID NO: 4, or modified from SEQ ID NO: 4 by a single alanine substitution at position 2, 3, 4, 5, 6, 8, 9, 10 or 11 or by one to five conservative amino acid substitutions at positions 2, 3, 4, 5, 6, 8, 9, 10, 11 and/or 12.

10. The isolated human antibody of claim 9, or an antigen-binding portion thereof, which dissociates from human  $\text{TNF}\alpha$  with a  $K_{\text{off}}$  rate constant of  $5 \times 10^{-4} \text{ s}^{-1}$  or less.

11. The isolated human antibody of claim 9, or an antigen-binding portion thereof, which dissociates from human  $\text{TNF}\alpha$  with a  $K_{\text{off}}$  rate constant of  $1 \times 10^{-4} \text{ s}^{-1}$  or less.

12. An isolated human antibody, or an antigen-binding portion thereof, with a light chain variable region (LCVR) having a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 3, or modified from SEQ ID NO: 3 by a single alanine substitution at position 1, 4, 5, 7 or 8, and with a heavy chain variable region (HCVR) having a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 4, or modified from SEQ ID NO: 4 by a single alanine substitution at position 2, 3, 4, 5, 6, 8, 9, 10 or 11.

13. The isolated human antibody, or an antigen-binding portion thereof, of claim 12, wherein the LCVR further has a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 5 and the HCVR further has a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 6.

14. The isolated human antibody, or an antigen-binding portion thereof, of claim 13, wherein the LCVR further has CDR1 domain comprising the amino acid sequence of SEQ ID NO: 7 and the HCVR has a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 8.

15. An isolated human antibody, or an antigen binding portion thereof, with a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO: 1 and a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 2.

16. The isolated human antibody of claim 15, which has an IgG1 heavy chain constant region.

17. The isolated human antibody of claim 15, which has an IgG4 heavy chain constant region.

18. The isolated human antibody of claim 15, which is a Fab fragment.

19. The isolated human antibody of claim 15, which is a single chain Fv fragment.

20. An isolated human antibody, or an antigen-binding portions thereof, with a light chain variable region (LCVR) having a CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26 or with a heavy chain variable region (HCVR) having a CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33 and SEQ ID NO: 34.

21. An isolated human antibody, or antigen-binding portion thereof, that neutralizes the activity of human TNF $\alpha$ , chimpanzee TNF $\alpha$  and at least one additional primate TNF $\alpha$  selected from the group consisting of baboon TNF $\alpha$ , marmoset TNF $\alpha$ , cynomolgus TNF $\alpha$  and rhesus TNF $\alpha$ .

22. The isolated human antibody, or an antigen-binding portion thereof, of claim 21, which also neutralizes the activity of mouse TNF $\alpha$ .

23. The isolated human antibody, or an antigen-binding portion thereof, of claim 21, which also neutralizes the activity of pig TNF $\alpha$ .

24. An isolated nucleic acid encoding a light chain CDR3 domain comprising the amino acid sequence of SEQ ID NO: 3, or modified from SEQ ID NO: 3 by a single alanine substitution at position 1, 4, 5, 7 or 8, or by one to five conservative amino acid substitutions at positions 1, 3, 4, 6, 7, 8 and/or 9.

25. The isolated nucleic acid of claim 24, which encodes an antibody light chain variable region (LCVR).

26. The isolated nucleic acid of claim 25, wherein the CDR2 domain of the antibody LCVR comprises the amino acid sequence of SEQ ID NO: 5.

27. The isolated nucleic acid of claim 26, wherein the CDR1 domain of the antibody LCVR comprises the amino acid sequence of SEQ ID NO: 7.

28. An isolated nucleic acid encoding a heavy chain CDR3 domain comprising the amino acid sequence of SEQ ID NO: 4, or modified from SEQ ID NO: 4 by a single alanine substitution at position 2, 3, 4, 5, 6, 8, 9, 10 or 11, or by one to five conservative amino acid substitutions at positions 2, 3, 4, 5, 6, 8, 9, 10, 11 and/or 12.

29. The isolated nucleic acid of claim 28, which encodes an antibody heavy chain variable region (HCVR).

30. The isolated nucleic acid of claim 29, wherein the CDR2 domain of the antibody HCVR comprises the amino acid sequence of SEQ ID NO: 6.

31. The isolated nucleic acid of claim 30, wherein the CDR1 domain of the antibody HCVR comprises the amino acid sequence of SEQ ID NO: 8.

32. An isolated nucleic acid encoding a CDR3 domain comprising an amino acid sequence selected from the group consisting of: SEQ ID NO: 3, SEQ ID NO 4, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33 and SEQ ID NO: 34.

33. An isolated nucleic acid encoding an antibody light chain variable region comprising the amino acid sequence of SEQ ID NO: 1.

34. The isolated nucleic acid of claim 33, which encodes the antibody light chain variable region and an antibody light chain constant region.

35. The isolated nucleic acid of claim 34, which is in a recombinant expression vector.

36. An isolated nucleic acid encoding an antibody heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 2.

37. The isolated nucleic acid of claim 36, which encodes the antibody heavy chain variable region and an antibody heavy chain constant region.

38. The isolated nucleic acid of claim 37, wherein the antibody heavy chain constant region is an IgG1 constant region.

39. The isolated nucleic acid of claim 37, wherein the antibody heavy chain constant region is an IgG4 constant region.

40. The isolated nucleic acid of claim 37, which is in a recombinant expression vector.

41. A recombinant expression vector encoding:

a) an antibody light chain having a variable region comprising the amino acid sequence of SEQ ID NO: 1; and

b) an antibody heavy chain having a variable region comprising the amino acid sequence of SEQ ID NO: 2.

42. A host cell into which the recombinant expression vector of claim 41 has been introduced.

43. A method of synthesizing a human antibody that binds human  $\text{TNF}\alpha$ , comprising culturing the host cell of claim 42 in a culture medium until a human antibody that binds human  $\text{TNF}\alpha$  is synthesized by the cell.

44. A pharmaceutical composition comprising the antibody of claim 1 and a pharmaceutically acceptable carrier.

45. A pharmaceutical composition comprising the antibody of claim 9 and a pharmaceutically acceptable carrier.

46. A pharmaceutical composition comprising the antibody of claim 15 and a pharmaceutically acceptable carrier.

47. A method for inhibiting human  $\text{TNF}\alpha$  activity comprising contacting human  $\text{TNF}\alpha$  with the antibody of claim 1 such that human  $\text{TNF}\alpha$  activity is inhibited.

48. A method for inhibiting human  $\text{TNF}\alpha$  activity comprising contacting human  $\text{TNF}\alpha$  with the antibody of claim 9 such that human  $\text{TNF}\alpha$  activity is inhibited.



49. A method for inhibiting human TNF $\alpha$  activity comprising contacting human TNF $\alpha$  with the antibody of claim 15 such that human TNF $\alpha$  activity is inhibited.

50. A method for inhibiting human TNF $\alpha$  activity in a human subject suffering from a disorder in which TNF $\alpha$  activity is detrimental, comprising administering to the human subject the antibody of claim 1 such that human TNF $\alpha$  activity in the human subject is inhibited.

51. A method for inhibiting human TNF $\alpha$  activity in a human subject suffering from a disorder in which TNF $\alpha$  activity is detrimental, comprising administering to the human subject the antibody of claim 9 such that human TNF $\alpha$  activity in the human subject is inhibited.

52. A method for inhibiting human TNF $\alpha$  activity in a human subject suffering from a disorder in which TNF $\alpha$  activity is detrimental, comprising administering to the human subject the antibody of claim 15 such that human TNF $\alpha$  activity in the human subject is inhibited.

53. The method of claim 52, wherein the disorder is sepsis.

54. The method of claim 53, wherein the antibody is administered to the human subject together with the cytokine interleukin-6 (IL-6) or is administered to a human subject with a serum or plasma concentration of IL-6 above 500 pg/ml.

55. The method of claim 52, wherein the disorder is an autoimmune disease.

56. The method of claim 55, wherein the autoimmune disease is selected from the group consisting of rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis and gouty arthritis.

57. The method of claim 55, wherein the autoimmune disease is selected from the group consisting of an allergy, multiple sclerosis, autoimmune diabetes, autoimmune uveitis and nephrotic syndrome.

58. The method of claim 52, wherein the disorder is an infectious disease.

59. The method of claim 52, wherein the disorder is transplant rejection or graft-versus-host disease.

60. The method of claim 52, wherein the disorder is a malignancy.
61. The method of claim 52, wherein the disorder is a pulmonary disorder.
- 5 62. The method of claim 52, wherein the disorder is an intestinal disorder.
63. The method of claim 52, wherein the disorder is a cardiac disorder.
64. The method of claim 52, wherein the disorder is selected from the group  
10 consisting of inflammatory bone disorders, bone resorption disease, alcoholic hepatitis, viral hepatitis, coagulation disturbances, burns, reperfusion injury, keloid formation, scar tissue formation and pyrexia.



## HUMAN ANTIBODIES THAT BIND HUMAN TNF $\alpha$

### Abstract

5

Human antibodies, preferably recombinant human antibodies, that specifically bind to human tumor necrosis factor  $\alpha$  (hTNF $\alpha$ ) are disclosed. These antibodies have high affinity for hTNF $\alpha$  (*e.g.*,  $K_d = 10^{-8}$  M or less), a slow off rate for hTNF $\alpha$  dissociation (*e.g.*,  $K_{off} = 10^{-3} \text{ sec}^{-1}$  or less) and neutralize hTNF $\alpha$  activity *in vitro* and *in vivo*. An antibody of the invention can be a full-length antibody or an antigen-binding portion thereof. The antibodies, or antibody portions, of the invention are useful for detecting hTNF $\alpha$  and for inhibiting hTNF $\alpha$  activity, *e.g.*, in a human subject suffering from a disorder in which hTNF $\alpha$  activity is detrimental. Nucleic acids, vectors and host cells for expressing the recombinant human antibodies of the invention, and methods of synthesizing the recombinant human antibodies, are also encompassed by the invention.

10

15

	CDR L1		CDR L2
2SD4 VL DIQMTQSPSSLSASIGDRVITIC	RASQGIRNYLA	WYQKPGKAPKLLIY	AASTLQS
EP B12	.....	.....	.....
VL10E4	.....	.....	.....
VL100A9	.....	.....	.....
VL100D2	.....	.....	.....
VL10F4	.....	.....	.....
LOE5	.....	.....	.....
VLL0F9	.....	.....	.....
VLL0F10	.....	.....	.....
VLL0G7	.....	.....	.....
VLL0G9	.....	.....	.....
VLL0H1	.....	.....	.....
VLL0H10	.....	.....	.....
VLLB7	.....	.....	.....
VLC1	.....	.....	.....
VLC7	.....	.....	.....
VLO1F4	.....	.....	.....
VLO1H8	.....	.....	.....
LOE7	.....	.....	.....
LOE7.A	.....	.....	.....
LOE7.T	.....	.....	.....
D2E7 VL DIQMTQSPSSLSASVGDVRTIC	RASQGIRNYLA	WYQKPGKAPKLLIY	AASTLQS
LD2E7* A1	.....	.....	.....
LD2E7* A3	.....	.....	.....
LD2E7* A4	.....	.....	.....
LD2E7* A5	.....	.....	.....
LD2E7* A7	.....	.....	.....
LD2E7* A8	.....	.....	.....

Figure 1A

1000

CDR L3	1 2 3 4 5 6 7 8 9	FGQGTKEIK
2SD4 VL	GVPSRFGSGSGTDFTLTISSLQPEDVATYYC	QKNSAPYA
EP B12	.....	.....R.....
VL10E4	.....	.....QR.....T
VL100A9	.....	.....QS.....T
VL100D2	.....	VL100D2.....T
VL10F4	.....	.....R.....T
LOE5	.....	.....Y.....S
VL10F9	.....	.....R.....R.....T
VL10F10	.....	.....T.....T.....P
VL10G7	.....	.....N.....N.....
VL10G9	.....	.....T.....T.....
VL10H1	.....	.....R.....N.....
VL10H10	.....	.....A.....S.....
VL1B7	.....	.....Q.....DT.....W
VL1C1	.....	.....D.....T.....
VL1C7	.....	.....N.....
VL0.1F4	.....	.....I.....T.....
VL0.1H8	.....	.....RP.....T.....R
LOE7	.....	.....R.....R.....T.....
LOE7.A	.....	.....R.....R.....
LOE7.T	.....	.....R.....R.....T.....
D2E7 VL	GVPSRFGSGSGTDFTLTISSLQPEDVATYYC	QYNRAPYT
LD2E7*.A1	.....	.....A.....
LD2E7*.A3	.....	.....A.....
LD2E7*.A4	.....	.....A.....
LD2E7*.A5	.....	.....A.....
LD2E7*.A7	.....	.....A.....
LD2E7*.A8	.....	.....A.....

### Figure 1B

CDR H1		CDR H2	
2SDA VH QVQLVESGGGLVQPGRSLRLSCAASGFTFD	DYAMH	WVRQAPGKGLDWVS	AITWNSCHIDYADSVEG
VH1B1 . . . . .	. . . . .	. . . . .	. . . . .
VH1D8 . . . . .	. . . . .	. . . . .	. . . . .
VH1A11 . . . . .	. . . . .	. . . . .	. . . . .
VH1B12 . . . . .	. . . . .	. . . . .	. . . . .
VH1-D2 . . . . .	. . . . .	. . . . .	. . . . .
VH1B4 . . . . .	. . . . .	. . . . .	. . . . .
VH1F6 . . . . .	. . . . .	. . . . .	. . . . .
VH1G1 . . . . .	. . . . .	. . . . .	. . . . .
3C-H2 . . . . .	. . . . .	. . . . .	. . . . .
VH1-D2.N . . . . .	. . . . .	. . . . .	. . . . .
VH1-D2.Y . . . . .	. . . . .	. . . . .	. . . . .
D2E7 VH EVQLVESGGGLVQPGRSLRLSCAASGFTFD	DYAMH	WVRQAPGKGLEWVS	AITWNSGHIDYADSVEG
HD2E7*.A1 . . . . .	. . . . .	. . . . .	. . . . .
HD2E7*.A2 . . . . .	. . . . .	. . . . .	. . . . .
HD2E7*.A3 . . . . .	. . . . .	. . . . .	. . . . .
HD2E7*.A4 . . . . .	. . . . .	. . . . .	. . . . .
HD2E7*.A5 . . . . .	. . . . .	. . . . .	. . . . .
HD2E7*.A6 . . . . .	. . . . .	. . . . .	. . . . .
HD2E7*.A7 . . . . .	. . . . .	. . . . .	. . . . .
HD2E7*.A8 . . . . .	. . . . .	. . . . .	. . . . .
HD2E7*.A9 . . . . .	. . . . .	. . . . .	. . . . .

Figure 2A

CDR H3

	1	2	3	4	5	6	7	8	9	10	11	12
2SP4 VH RFAVSRDNAKNALYLQMNLSRPEDTAVYVCTK	A	S	Y	L	S	T	S	S	L	D	N	
VH1B11	.	.	.	.	.	.	.	.	.	.	.	K
VH1D8	.	.	.	.	.	.	.	.	.	.	.	Y
VH1A11	.	.	.	.	.	.	.	.	.	.	.	D
VH1B12	.	.	.	.	.	.	.	.	.	.	.	F
VH1-D2	.	.	.	.	.	.	.	.	.	.	.	Y
VH1E4	.	.	.	.	.	.	.	.	.	.	.	A
VH1F6	.	.	.	.	.	.	.	.	.	.	.	Y
VH1G1	.	.	.	.	.	.	.	.	.	.	.	Y
3C-H2	.	.	.	.	.	.	.	.	.	.	.	Y
VH1-D2.N	.	.	.	.	.	.	.	.	.	.	.	N
VH1-D2.Y	.	.	.	.	.	.	.	.	.	.	.	Y
D2E7 VH RFTISRDNAKNSLYLQMNLSRAEDTAVYVCAK	V	S	Y	L	S	T	A	S	S	L	D	Y
HD2E7* A1	.	.	.	.	.	.	.	.	.	.	.	A
HD2E7* A2	.	.	.	.	.	.	.	.	.	.	.	A
HD2E7* A3	.	.	.	.	.	.	.	.	.	.	.	A
HD2E7* A4	.	.	.	.	.	.	.	.	.	.	.	A
HD2E7* A5	.	.	.	.	.	.	.	.	.	.	.	A
HD2E7* A6	.	.	.	.	.	.	.	.	.	.	.	A
HD2E7* A7	.	.	.	.	.	.	.	.	.	.	.	A
HD2E7* A8	.	.	.	.	.	.	.	.	.	.	.	A
HD2E7* A9	.	.	.	.	.	.	.	.	.	.	.	A
	W	C	Q	G	T	L	V	T	V	S	S	

Figure 2B

FIG. 3

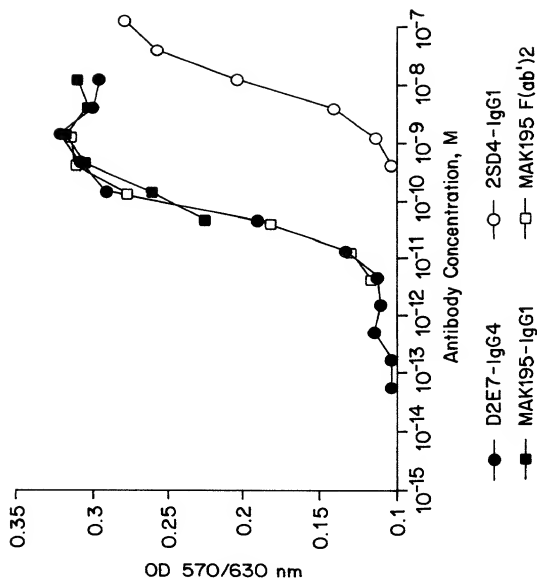




FIG. 4

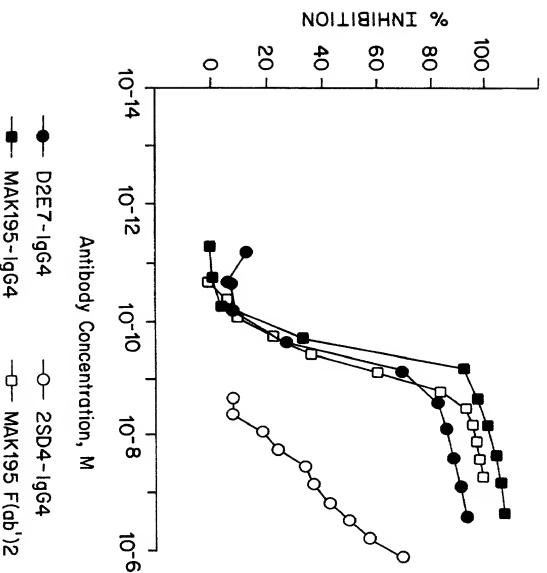


FIG. 5

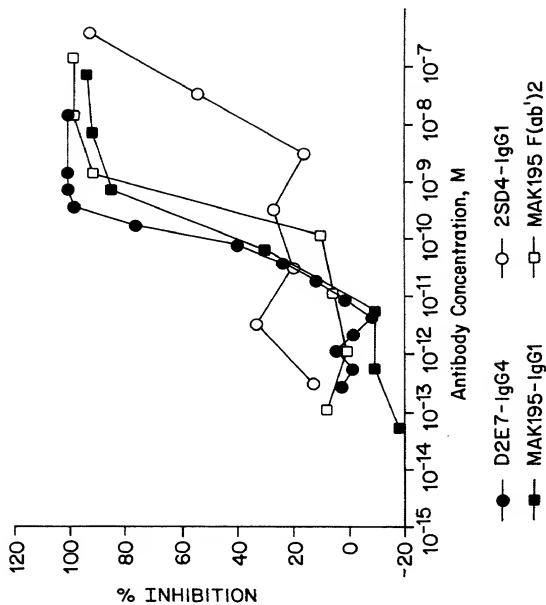
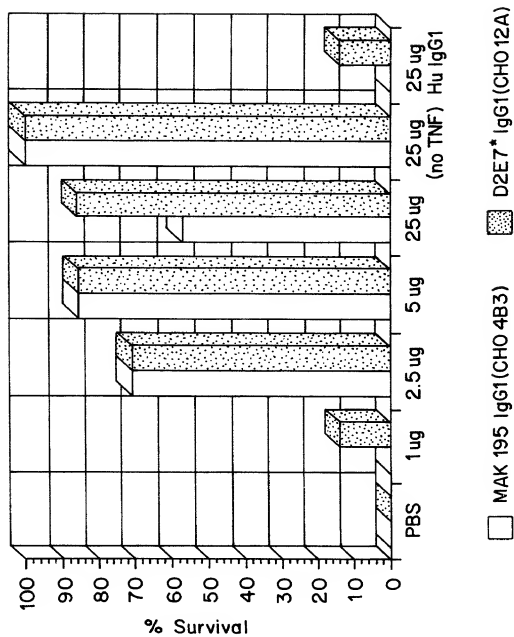


FIG. 6



D2E7 VL

GAC ATC CAG ATG ACC CAG TCT CCA TCC TCC CTG TCT GCA TCT GTA  
D I Q M T Q S P S S L S A S V

CDR L1

GGG GAC AGA GTC ACC ATC ACT TGT CGG GCA AGT CAG GGC ATC AGA  
G D R V T I T C R A S Q G I R

AAT TAC TTA GCC TGG TAT CAG CAA AAA CCA GGG AAA GCC CCT AAG  
N Y L A W Y Q Q K P G K A P K

CDR L2

CTC CTG ATC TAT GCT GCA TCC ACT TTG CAA TCA GGG GTC CCA TCT  
L L I Y A A S T L Q S G V P S

CGG TTC AGT GGC AGT GGA TCT GGG ACA GAT TTC ACT CTC ACC ATC  
R F S G S G S G T D F T L T I

AGC AGC CTA CAG CCT GAA GAT GTT GCA ACT TAT TAC TGT CAA AGG  
S S L Q P E D V A T Y Y C Q R

CDR L3

TAT AAC CGT GCA CCG TAT ACT TTT GGC CAG GGG ACC AAG GTG GAA  
Y N R A P Y T F G Q G T K V E

ATC AAA  
I K

FIGURE 7

D2E7 VH

GAG GTG CAG CTG GTG GAG TCT GGG GGA GGC TTG GTA CAG CCC GGC  
E V Q L V E S G G G L V Q P G

AGG TCC CTG AGA CTC TCC TGT GCG GCC TCT GGA TTC ACC TTT GAT  
R S L R L S C A A S G F T F D

**CDR H1**

GAT TAT GCC ATG CAC TGG GTC CGG CAA GCT CCA GGG AAG GGC CTG  
D Y A M H W V R Q A P G K G L

**CDR H2**

GAA TGG GTC TCA GCT ATC ACT TGG AAT AGT GGT CAC ATA GAC TAT  
E W V S A I T W N S G H I D Y

GCG GAC TCT GTG GAG GGC CGA TTC ACC ATC TCC AGA GAC AAC GCC  
A D S V E G R F T I S R D N A

AAG AAC TCC CTG TAT CTG CAA ATG AAC AGT CTG AGA GCT GAG GAT  
K N S L Y L Q M N S L R A E D

**CDR H3**

ACG GCC GTA TAT TAC TGT GCG AAA GTC TCG TAC CTT AGC ACC GCG  
T A V Y Y C A K V S Y L S T A

TCC TCC CTT GAC TAT TGG GGC CAA GGT ACC CTG GTC ACC GTC TCG  
S S L D Y W G Q G T L V T V S

AGT  
S

FIGURE 8

Declaration, Petition and Power of Attorney For Patent Application

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

**HUMAN ANTIBODIES THAT BIND HUMAN TNF $\alpha$**

the specification of which

(check one)

☐ is attached hereto.

☒ was filed on February 9, 1996 as

Application Serial No. 08/599,226

and was amended on \_\_\_\_\_  
(if applicable)

I do not know and do not believe that the subject matter of this application was ever known or used in the United States before my invention thereof or patented or described in any printed publication in any country before my invention thereof or more than one year prior to the date of this application, and that said subject matter has not been patented or made the subject of an issued inventor's certificate in any country foreign to the United States on an application filed by me or my legal representatives or assigns more than twelve months prior to the date of this application; that I acknowledge my duty to disclose information of which I am aware which is material to the examination of this application, that no application for patent or inventor's certificate on the subject matter of this application has been filed by me or my representatives or assigns in any country foreign to the United States, except those identified below, and that I have reviewed and understand the contents of the specification, including the claims as amended by any amendment referred to herein.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

# PRIORITY CLAIM

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed.

Check one:

☒ no such applications have been filed.

☐ such applications have been filed as follows

EARLIEST FOREIGN APPLICATION(S), IF ANY FILED WITHIN 12 MONTHS  
(6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

Country	Application Number	Date of Filing (month,day,year)	Priority Claimed Under 35 USC 119
			<input type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No

ALL FOREIGN APPLICATION(S), IF ANY FILED MORE THAN 12 MONTHS  
(6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION


# CLAIM FOR BENEFIT OF EARLIER U.S./PCT APPLICATION(S)

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application. As to subject matter of this application which is common to my earlier United States application, if any, described below, I do not believe that the same was ever known or used in the United States before my invention thereof or patented or described in any printed publication in any country before my invention thereof or more than one year prior to said earlier application, or in public use or on sale in the United States more than one year prior to said earlier application, that the said common subject matter has not been patented or made the subject of an inventor's certificate issued before the date of said earlier application in any country foreign to the United States on an application, filed by me or my legal representatives or assigns more than twelve months prior to said application and that no application for patent or inventor's certificate on said subject matter has been filed by me or my representatives or assigns in any country foreign to the United States except those identified herein.

(Application Serial No.)

(Filing Date)

(Status)  
(patented,pending,aband.)

(Application Serial No.)

(Filing Date)

(Status)  
(patented,pending,aband.)



POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorneys and/or agents to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

John A. Lahive, Jr.	Reg. No. 19,788	John V. Bianco	Reg. No. 36,748
W. Hugo Liepmann	Reg. No. 20,407	Jeremiah Lynch	Reg. No. 17,425
James E. Cockfield	Reg. No. 19,162	Amy E. Mandragouras	Reg. No. 36,207
Thomas V. Smurzynski	Reg. No. 24,798	Elizabeth A. Hanley	Reg. No. 33,505
Ralph A. Loren	Reg. No. 29,325	Anthony A. Laurentano	Reg. No. 38,220
Thomas J. Engellenner	Reg. No. 28,711	Jane E. Remillard	Reg. No. 38,872
William C. Geary III	Reg. No. 31,359	Edward J. Kelly	Reg. No. 38,936
Giulio A. DeConti, Jr.	Reg. No. 31,503	Mark A. Kurisko	Reg. No. 38,944
Ann Lamport Hammitte	Reg. No. 34,858	Beth E. Arnold	Reg. No. 35,430
Paul Louis Myers	Reg. No. 35,965	Jean M. Silveri	Reg. No. 39,030
Michael I. Falkoff	Reg. No. 30,833	Matthew P. Vincent	Reg. No. 36,709

and to: Joseph D. Michaels, Reg. No. 22,737  
BASF Bioresearch Corporation  
100 Research Drive  
Worcester, MA 01605-4314

Send Correspondence to:

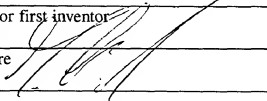
Lahive & Cockfield, 60 State Street, Suite 510, Boston, MA 02109-1875

Direct Telephone Calls to: (name and telephone number)

Giulio A. DeConti, Jr., Esq., (617) 227-7400

Wherefore I petition that letters patent be granted to me for the invention or discovery described and claimed in the attached specification and claims, and hereby subscribe my name to said specification and claims and to the foregoing declaration, power of attorney, and this petition.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

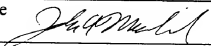
Full name of sole or first inventor Jochen G. Salfeld	
Inventor's signature 	Date 4/29/96
Residence 177 Old Westboro Road, North Grafton, MA	
Citizenship Germany	
Post Office Address (if different)	

Full name of second inventor, if any Deborah J. Allen	
Inventor's signature	Date
Residence 143a Shelbourne Road London N17 9YD	
Citizenship Great Britain	
Post Office Address (if different)	

Full name of third inventor, if any Hendricus R.J.M. Hoogenboom	
Inventor's signature	Date
Residence Muggenstraat 45 Bus 12, Hasselt B3500 Belgium	
Citizenship The Netherlands	
Post Office Address (if different)	

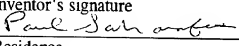
Full name of fourth inventor, if any Zehra Kaymakalan	
Inventor's signature <i>Zehra Kaymakalan</i>	Date 4/22/96
Residence 4 Piccadilly Way, Westboro, MA 01581	
Citizenship Turkey	
Post Office Address (if different)	

Full name of fifth inventor, if any Boris Labkovsky	
Inventor's signature <i>Boris Labkovsky</i>	Date 4-22-96
Residence 1630 Worcester Road, Apt. 532, Framingham, MA 01701	
Citizenship U.S.A.	
Post Office Address (if different)	

Full name of sixth inventor, if any John A. Mankovich	
Inventor's signature 	Date 4-22-96
Residence 416 Lowell Street, Andover, MA 01810	
Citizenship U.S.A.	
Post Office Address (if different)	

Full name of seventh inventor, if any Brian T. McGuinness	
Inventor's signature	Date
Residence 22 The Lane, Hauxton, Cambridge CB2 5HP	
Citizenship Great Britain	
Post Office Address (if different)	

Full name of eighth inventor, if any Andrew J. Roberts	
Inventor's signature	Date
Residence 15 Cavendish Road, Cambridge CB1 3AE	
Citizenship Great Britain	
Post Office Address (if different)	

Full name of ninth inventor, if any Paul Sakorafas	
Inventor's signature 	Date 4-18-96
Residence 6114 Arbor Drive, Shrewsbury, MA 01545	
Citizenship U.S.A.	
Post Office Address (if different)	

Full name of tenth inventor, if any David Schoenhaut	
Inventor's signature	Date
Residence 55 East Ninth Street, Clifton, NJ 07011	
Citizenship U.S.A.	
Post Office Address (if different)	

Full name of eleventh inventor, if any Tristan J. Vaughan	
Inventor's signature	Date
Residence 9 Villa Road, Impington, Cambridge, CB4 4NZ	
Citizenship Great Britain	
Post Office Address (if different)	

Full name of twelfth inventor, if any Michael White	
Inventor's signature <i>Michael K White</i>	Date 7/31/96
Residence 30 Angelica Drive, Framingham, MA 01701	
Citizenship U.S.A.	
Post Office Address (if different)	

Full name of thirteenth inventor, if any Alison J. Wilton	
Inventor's signature	Date
Residence 46 Huntingdon Road, Cambridge, CB3 0HH	
Citizenship Great Britain	
Post Office Address (if different)	

Declaration, Petition and Power of Attorney For Patent Application

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

**HUMAN ANTIBODIES THAT BIND HUMAN TNF $\alpha$**

the specification of which

(check one)

☐ is attached hereto.

☒ was filed on February 9, 1996 as

Application Serial No. 08/599,226

and was amended on \_\_\_\_\_  
(if applicable)

I do not know and do not believe that the subject matter of this application was ever known or used in the United States before my invention thereof or patented or described in any printed publication in any country before my invention thereof or more than one year prior to the date of this application, and that said subject matter has not been patented or made the subject of an issued inventor's certificate in any country foreign to the United States on an application filed by me or my legal representatives or assigns more than twelve months prior to the date of this application; that I acknowledge my duty to disclose information of which I am aware which is material to the examination of this application, that no application for patent or inventor's certificate on the subject matter of this application has been filed by me or my representatives or assigns in any country foreign to the United States, except those identified below, and that I have reviewed and understand the contents of the specification, including the claims as amended by any amendment referred to herein.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

# PRIORITY CLAIM

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed.

Check one:

☒ no such applications have been filed.

☐ such applications have been filed as follows

EARLIEST FOREIGN APPLICATION(S), IF ANY FILED WITHIN 12 MONTHS  
(6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

Country	Application Number	Date of Filing (month,day,year)	Priority Claimed Under 35 USC 119
			<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/>
			<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/>
			<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/>
			<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/>
			<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/>

ALL FOREIGN APPLICATION(S), IF ANY FILED MORE THAN 12 MONTHS  
(6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION


# CLAIM FOR BENEFIT OF EARLIER U.S./PCT APPLICATION(S)

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application. As to subject matter of this application which is common to my earlier United States application, if any, described below, I do not believe that the same was ever known or used in the United States before my invention thereof or patented or described in any printed publication in any country before my invention thereof or more than one year prior to said earlier application, or in public use or on sale in the United States more than one year prior to said earlier application, that the said common subject matter has not been patented or made the subject of an inventor's certificate issued before the date of said earlier application in any country foreign to the United States on an application, filed by me or my legal representatives or assigns more than twelve months prior to said application and that no application for patent or inventor's certificate on said subject matter has been filed by me or my representatives or assigns in any country foreign to the United States except those identified herein.

(Application Serial No.)

(Filing Date)

(Status)  
(patented,pending,aband.)

(Application Serial No.)

(Filing Date)

(Status)  
(patented,pending,aband.)

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorneys and/or agents to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

John A. Lahive, Jr.	Reg. No. 19,788	John V. Bianco	Reg. No. 36,748
W. Hugo Liepmann	Reg. No. 20,407	Jeremiah Lynch	Reg. No. 17,425
James E. Cockfield	Reg. No. 19,162	Amy E. Mandragouras	Reg. No. 36,207
Thomas V. Smurzynski	Reg. No. 24,798	Elizabeth A. Hanley	Reg. No. 33,505
Ralph A. Loren	Reg. No. 29,325	Anthony A. Laurentano	Reg. No. 38,220
Thomas J. Engellenner	Reg. No. 28,711	Jane E. Remillard	Reg. No. 38,872
William C. Geary III	Reg. No. 31,359	Edward J. Kelly	Reg. No. 38,936
Giulio A. DeConti, Jr.	Reg. No. 31,503	Mark A. Kurisko	Reg. No. 38,944
Ann Lamport Hammitte	Reg. No. 34,858	Beth E. Arnold	Reg. No. 35,430
Paul Louis Myers	Reg. No. 35,965	Jean M. Silveri	Reg. No. 39,030
Michael I. Falkoff	Reg. No. 30,833	Matthew P. Vincent	Reg. No. 36,709

and to: Joseph D. Michaels, Reg. No. 22,737  
BASF Bioresearch Corporation  
100 Research Drive  
Worcester, MA 01605-4314

Send Correspondence to:

Lahive & Cockfield, 60 State Street, Suite 510, Boston, MA 02109-1875

Direct Telephone Calls to: (name and telephone number)

Giulio A. DeConti, Jr., Esq., (617) 227-7400

Wherefore I petition that letters patent be granted to me for the invention or discovery described and claimed in the attached specification and claims, and hereby subscribe my name to said specification and claims and to the foregoing declaration, power of attorney, and this petition.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first inventor Jochen G. Salfeld	
Inventor's signature	Date
Residence 177 Old Westboro Road, North Grafton, MA	
Citizenship Germany	
Post Office Address (if different)	



Full name of second inventor, if any Deborah J. Allen	
Inventor's signature	Date
Residence 143a Shelbourne Road London N17 9YD	
Citizenship Great Britain	
Post Office Address (if different)	

Full name of third inventor, if any Hendricus R.J.M. Hoogenboom	
Inventor's signature	Date
Residence Muggenstraat 45 Bus 12, Hasselt B3500 Belgium	
Citizenship The Netherlands	
Post Office Address (if different)	

Full name of fourth inventor, if any Zehra Kaymakcalan	
Inventor's signature	Date
Residence 4 Piccadilly Way, Westboro, MA 01581	
Citizenship Turkey	
Post Office Address (if different)	

Full name of fifth inventor, if any Boris Labkovsky	
Inventor's signature	Date
Residence 1630 Worcester Road, Apt. 532, Framingham, MA 01701	
Citizenship U.S.A.	
Post Office Address (if different)	

Full name of sixth inventor, if any John A. Mankovich	
Inventor's signature	Date
Residence 416 Lowell Street, Andover, MA 01810	
Citizenship U.S.A.	
Post Office Address (if different)	

Full name of seventh inventor, if any Brian T. McGuinness	
Inventor's signature	Date
Residence 22 The Lane, Hauxton, Cambridge CB2 5HP	
Citizenship Great Britain	
Post Office Address (if different)	

Full name of eighth inventor, if any Andrew J. Roberts	
Inventor's signature	Date
Residence 15 Cavendish Road, Cambridge CB1 3AE	
Citizenship Great Britain	
Post Office Address (if different)	

Full name of ninth inventor, if any Paul Sakorafas	
Inventor's signature	Date
Residence 6114 Arbor Drive, Shrewsbury, MA 01545	
Citizenship U.S.A.	
Post Office Address (if different)	

Full name of tenth inventor, if any David Schoenhaut	
Inventor's signature <i>David Schoenhaut</i>	Date 4/24/96
Residence 55 East Ninth Street, Clifton, NJ 07011	
Citizenship U.S.A.	
Post Office Address (if different)	

Full name of eleventh inventor, if any Tristan J. Vaughan	
Inventor's signature	Date
Residence 9 Villa Road, Impington, Cambridge, CB4 4NZ	
Citizenship Great Britain	
Post Office Address (if different)	

Full name of twelfth inventor, if any Michael White	
Inventor's signature	Date
Residence 30 Angelica Drive, Framingham, MA 01701	
Citizenship U.S.A.	
Post Office Address (if different)	

Full name of thirteenth inventor, if any Alison J. Wilton	
Inventor's signature	Date
Residence 46 Huntingdon Road, Cambridge, CB3 0HH	
Citizenship Great Britain	
Post Office Address (if different)	

Declaration, Petition and Power of Attorney For Patent Application

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

**HUMAN ANTIBODIES THAT BIND HUMAN TNF $\alpha$**

the specification of which

(check one)

☐ is attached hereto.

☒ was filed on February 9, 1996 as

Application Serial No. 08/599,226

and was amended on \_\_\_\_\_  
(if applicable)

I do not know and do not believe that the subject matter of this application was ever known or used in the United States before my invention thereof or patented or described in any printed publication in any country before my invention thereof or more than one year prior to the date of this application, and that said subject matter has not been patented or made the subject of an issued inventor's certificate in any country foreign to the United States on an application filed by me or my legal representatives or assigns more than twelve months prior to the date of this application; that I acknowledge my duty to disclose information of which I am aware which is material to the examination of this application, that no application for patent or inventor's certificate on the subject matter of this application has been filed by me or my representatives or assigns in any country foreign to the United States, except those identified below, and that I have reviewed and understand the contents of the specification, including the claims as amended by any amendment referred to herein.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

# PRIORITY CLAIM

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed.

Check one:

☒ no such applications have been filed.

☐ such applications have been filed as follows

EARLIEST FOREIGN APPLICATION(S), IF ANY FILED WITHIN 12 MONTHS  
(6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

Country	Application Number	Date of Filing (month,day,year)	Priority Claimed Under 35 USC 119
			<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/>
			<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/>
			<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/>
			<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/>
			<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/>

ALL FOREIGN APPLICATION(S), IF ANY FILED MORE THAN 12 MONTHS  
(6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION


# CLAIM FOR BENEFIT OF EARLIER U.S./PCT APPLICATION(S)

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application. As to subject matter of this application which is common to my earlier United States application, if any, described below, I do not believe that the same was ever known or used in the United States before my invention thereof or patented or described in any printed publication in any country before my invention thereof or more than one year prior to said earlier application, or in public use or on sale in the United States more than one year prior to said earlier application, that the said common subject matter has not been patented or made the subject of an inventor's certificate issued before the date of said earlier application in any country foreign to the United States on an application, filed by me or my legal representatives or assigns more than twelve months prior to said application and that no application for patent or inventor's certificate on said subject matter has been filed by me or my representatives or assigns in any country foreign to the United States except those identified herein.

(Application Serial No.)

(Filing Date)

(Status)  
(patented,pending,aband.)

(Application Serial No.)

(Filing Date)

(Status)  
(patented,pending,aband.)

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorneys and/or agents to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

John A. Lahive, Jr.	Reg. No. 19,788	John V. Bianco	Reg. No. 36,748
W. Hugo Liepmann	Reg. No. 20,407	Jeremiah Lynch	Reg. No. 17,425
James E. Cockfield	Reg. No. 19,162	Amy E. Mandragouras	Reg. No. 36,207
Thomas V. Smurzynski	Reg. No. 24,798	Elizabeth A. Hanley	Reg. No. 33,505
Ralph A. Loren	Reg. No. 29,325	Anthony A. Laurentano	Reg. No. 38,220
Thomas J. Engellerner	Reg. No. 28,711	Jane E. Remillard	Reg. No. 38,872
William C. Geary III	Reg. No. 31,359	Edward J. Kelly	Reg. No. 38,936
Giulio A. DeConti, Jr.	Reg. No. 31,503	Mark A. Kurisko	Reg. No. 38,944
Ann Lamport Hammitte	Reg. No. 34,858	Beth E. Arnold	Reg. No. 35,430
Paul Louis Myers	Reg. No. 35,965	Jean M. Silveri	Reg. No. 39,030
Michael I. Falkoff	Reg. No. 30,833	Matthew P. Vincent	Reg. No. 36,709

and to: Joseph D. Michaels, Reg. No. 22,737  
BASF Bioresearch Corporation  
100 Research Drive  
Worcester, MA 01605-4314

Send Correspondence to:

Lahive & Cockfield, 60 State Street, Suite 510, Boston, MA 02109-1875

Direct Telephone Calls to: (name and telephone number)

Giulio A. DeConti, Jr., Esq., (617) 227-7400

Wherefore I petition that letters patent be granted to me for the invention or discovery described and claimed in the attached specification and claims, and hereby subscribe my name to said specification and claims and to the foregoing declaration, power of attorney, and this petition.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first inventor	
Jochen G. Salfeld	
Inventor's signature	Date
Residence	
177 Old Westboro Road, North Grafton, MA	
Citizenship	
Germany	
Post Office Address (if different)	

Full name of second inventor, if any Deborah J. Allen	
Inventor's signature <i>Deborah J. Allen</i>	Date 23/4/9
Residence 143a Shelbourne Road London N17 9YD	
Citizenship Great Britain	
Post Office Address (if different)	

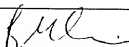
Full name of third inventor, if any Hendricus R.J.M. Hoogenboom	
Inventor's signature <i>H. Hoogenboom</i>	Date 17th April 196
Residence Muggenstraat 45 Bus 12, Hasselt B3500 Belgium	
Citizenship The Netherlands	
Post Office Address (if different)	

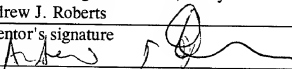
Full name of fourth inventor, if any Zehra Kaymakalan	
Inventor's signature	Date
Residence 4 Piccadilly Way, Westboro, MA 01581	
Citizenship Turkey	
Post Office Address (if different)	

Full name of fifth inventor, if any Boris Labkovsky	
Inventor's signature	Date
Residence 1630 Worcester Road, Apt. 532, Framingham, MA 01701	
Citizenship U.S.A.	
Post Office Address (if different)	



Full name of sixth inventor, if any John A. Mankovich	
Inventor's signature	Date
Residence 416 Lowell Street, Andover, MA 01810	
Citizenship U.S.A.	
Post Office Address (if different)	

Full name of seventh inventor, if any Brian T. McGuinness	
Inventor's signature 	Date 23/04/96
Residence 22 The Lane, Hauxton, Cambridge CB2 5HP	
Citizenship Great Britain	
Post Office Address (if different)	

Full name of eighth inventor, if any Andrew J. Roberts	
Inventor's signature 	Date 23/04/96
Residence 15 Cavendish Road, Cambridge CB1 3AE	
Citizenship Great Britain	
Post Office Address (if different)	

Full name of ninth inventor, if any Paul Sakorafas	
Inventor's signature	Date
Residence 6114 Arbor Drive, Shrewsbury, MA 01545	
Citizenship U.S.A.	
Post Office Address (if different)	

Full name of tenth inventor, if any David Schoenhaut	
Inventor's signature	Date
Residence 55 East Ninth Street, Clifton, NJ 07011	
Citizenship U.S.A.	
Post Office Address (if different)	

Full name of eleventh inventor, if any Tristan J. Vaughan	
Inventor's signature <i>Tristan J. Vaughan</i>	Date 23/04/96
Residence 9 Villa Road, Impington, Cambridge, CB4 4NZ	
Citizenship Great Britain	
Post Office Address (if different)	

Full name of twelfth inventor, if any Michael White	
Inventor's signature	Date
Residence 30 Angelica Drive, Framingham, MA 01701	
Citizenship U.S.A.	
Post Office Address (if different)	

Full name of thirteenth inventor, if any Alison J. Wilton	
Inventor's signature <i>Alison J. Wilton</i>	Date 23/4/96
Residence 46 Huntingdon Road, Cambridge, CB3 0HH	
Citizenship Great Britain	
Post Office Address (if different)	